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PROGRESS

SOME RECENT ADVANCES IN DERMATOLOGIC THERAPY

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THE material for the present summary has been gathered from the literature of the last two years. Selection has been entirely arbitrary, and many contributions of undoubted value have had to be omitted. Reference is made to some purely experimental work, not because it represents a practical advance in present dermatology, but because it may be the source of future progress in therapy.

VITAMIN THERAPY

The dermatologist deals primarily with the skin, the largest and most accessible organ of the human body. No other single organ offers such scope for the study of morphology and function in both health and disease. No other organ is subject to external assault by physical, chemical, mechanical, and biological agents; and at the same time, to internal onslaught by almost all the factors causing disease in more sheltered tissues. The dermatologist must, therefore, be an alert "internist" as well as a competent "externist." In many so-called "systemic diseases" a careful study of the skin and orificial mucous membranes will offer either supportive or conclusive diagnostic evidence (criteria). The avitaminoses (or "dysvitaminoses" as we prefer to call them) are a characteristic group in this category. However, the significance of the dermatologic approach to the study and management of vitamin deficiency is twofold. In the first place, it embraces the recognition of the classic general as well as local cutaneous manifestations of the various deficiency syndromes; in the second place, it includes the administration of vitamins to produce therapeutic effects on cutaneous lesions, as well as in order to achieve systemic effects or to build general resistance.

It is our opinion that many cases of vitamin "deficiencies" are not true deficiencies, but rather abnormal conditions of demand, utilization, and transport of the respective vitamins, "dysvitaminoses" (Sulzberger[1]). Moreover,

in numerous instances, it seems as though improvement takes place faster if several, and not only one, of the vitamins are administered. However, it is, of course, usually necessary to give that particular vitamin or complex which is most obviously needed in dosage much higher than that of the other vitamins.

Goodman, Kanof, Baer, and one of us (M. B. S.)[2] have shown that some cases of keratosis pilaris, formes trustes of ichthyosis, lichen spinulosus with or without cicatrizing alopecia, and brittleness of the nails are benefitted by large doses of *vitamin A*.

Probably the most striking of the newer studies on the role of vitamin A in dermatology is contained in the observations of Peck, Chargin, and Sobotka[3] on keratosis follicularis (Darier's disease). These investigators found that individuals with Darier's disease had a normal or increased amount of the provitamin A (carotene) in their blood serum, but a decidedly lower than normal ratio of vitamin A itself. These determinations were made by use of the photelometer,* with which quantitative estimations of vitamin A in blood serum are made according to the method of Carr and Price.[4] The estimations were confirmed by the dark adaptation method of Hecht and Mandelbaum.[5] It was further found that the dyskeratosis gradually subsided when serum vitamin A levels were raised to normal by the administration of large amounts (200,000 I.U. daily) of vitamin A concentrate, and recurred when this treatment was stopped or the dosage reduced. On the basis of this preliminary work, Peck and his collaborators believe Darier's disease to be due to an hereditary physiologic weakness in the absorption of vitamin A or in its conversion from the provitamin.

Recently, also, the dermatologic condition known as pityriasis rubra pilaris has been successfully treated by vitamin A in large doses. Brunsting and Sheard[6] had previously found that dark adaptation tests were similar to those later reported by Peck and co-workers in Darier's disease, and improvement in the dark adaptation which followed vitamin A therapy was, to some degree, paralleled by clinical improvement in the dermatosis. In this report, attention is drawn to the impairment of dark adaptation sometimes found associated with cirrhosis of the liver.[7] Recently, cases have been reported of the beneficial effect of vitamin B complex on pityriasis rubra pilaris.[8] This observation may lead to the assumption that the vitamin B complex produces an improvement in liver function which in turn permits the proper utilization of vitamin A, or the conversion of provitamin A to vitamin A. The reviewers have used vitamin A with some success in cases of phrynoderma, in individuals with hypothyroid habitus, and in various forms of "dryness of the skin," of lichen pilaris, of mild ichthyosis, and of follicular keratoses; and in patients giving a history of long-continued ingestion of mineral oil or of prolonged indulgence in some dietary fad. Debilitated or elderly patients with chronic dyskeratotic disease, or those suffering with chronic ulcers often respond to large doses of vitamin A, preferably complemented by B complex.

The *vitamin B complex* is justly receiving a lion's share of attention at present. It is our belief that, with a few notable exceptions, such as pellagra and polyneuritis, the vitamin B complex components are most effectively admin-

*Manufactured by the Central Scientific Co., Chicago, Ill.

istered as injections of whole crude liver extract (not the refined or concentrated preparations). This form is cheaper and much more efficacious than the 2 to 15 units per cubic centimeter products often employed. In many cases liver injections seem to be more effective also than much higher dosage of the present known vitamin B factors given by mouth. This may be due (1) to an unknown factor present in the crude liver; or (2) to a synergistic action of the various fractions of liver when given parenterally; or (3) to an alteration in composition of the concentrated or crystalline products produced by pharmaceutic houses for oral administration; or (4) to a faulty or selective alimentary absorption of the oral medication.

Madden[9] reports vitamin B_1 to be a valuable adjunct in the therapy of psoriasis. There is a growing literature on vitamin B_1 therapy in pink disease (acrodynia) in children.[10] Its use is well known in beriberi, and in the peripheral neuropathies of pellagra, alcoholism, and pregnancy.

Riboflavin (vitamin B_2) deficiency is characterized by some combination of the following signs and symptoms: redness of the buccal surface of the lips and abnormal redness of the vermilion borders with desquamation. There is usually maceration and fissuring, and sometimes rhagades-like lesions at the commissures. Seborrhea of the naso-labial folds, alae nasi, eyelids, and ears is frequent. There may be a specific type of glossitis with a purple-red color, fissuring, and coarse granular appearance. Conjunctival, scleral, and corneal injection and vascularization may be seen. The patient complains of burning of the eyeballs, roughening of the lids, visual fatigue, and photophobia.[11] All these conditions are promptly remedied by specific treatment—except the visual disturbances in long-standing cases. In dermatologic practice two points are worthy of stress in connection with the recent literature on riboflavin: the first is that *ordinary perlèche,* as occurring in children, or after trauma (dentist) and as seen so commonly by skin specialists, *is not a riboflavin deficiency and does not respond to riboflavin therapy.* The second point to be made is that while many of the corneal ulcers of *rosacea keratitis* respond to vitamin B_2, the cutaneous acne and rosacea remain unaffected, and do not, as a rule, respond to riboflavin.

Smith, Smith, and Callaway[12] describe a skin condition in pellagrins which they term "dyssebacia." The lesions, chiefly on the face, are characterized by dryness of the skin and the presence of plugs of inspissated sebum projecting from the sebaceous follicles. Dyssebacia is readily cured by autoclaved yeast by mouth or crude liver extract by injection, but is uninfluenced by oral administration of vitamin B_1 and liver extract. Nicotinic acid is effective but requires a much longer time than yeast or crude extract of liver.

The role of *nicotinic acid* in pellagra therapy is well known, as are its supposed and reported effects in alleviating some of the disagreeable subjective effects in sulfonamide therapy. The reviewers believe it may exert some beneficial effect in some cases of lupus erythematosus, perhaps by reducing the photosensitivity in this disease. They believe also that some patients tolerate oral bismuth (sobisminol) better while taking nicotinic acid, having less tendency to stomatitis and fewer gastric upsets. King[13] reports the disappearance of organisms of Vincent's angina in forty-eight to seventy-two hours with nicotinic acid therapy.

Vincent's angina and stomatitis are important, and are at present causing *much disability in military concentrations and defense projects,* and King's findings should merit further study.

The status of *pantothenic acid, biotin (vitamin H),* and *other factors* of the vitamin B complex has not been settled. A very thought-stimulating paper by György[14] discusses the possible relationship of these factors (as well as thiamine and riboflavin) to the scaly desquamative dermatoses in man. He has experimentally produced and cured in animals conditions similar to those of the "seborrheic diathesis" in man.

Spies[15] points out that pantothenic acid and riboflavin are mutually complementary, and believes the former to be a dietary necessity.

Kristensen and Vendel[16] claim to have effected a prompt, universal, and permanent clearing in 13 acute and 7 chronic cases of eczema treated with the vitamin B complex.

The Plummer-Vinson syndrome, characterized by anemia, dysphagia, glossitis, cheilitis, and spoon nails, is considered a precancerosis, probably related to vitamin B complex deficiency.[17] Notable among other suggested uses of vitamin B complex are its use in the treatment of (1) glossitis and stomatitis accompanying sprue, pernicious anemia, and pellagra;[17] (2) certain cases of neurodermatitis of the back and sides of neck and the elbow flexures;[17] (3) certain cases of moniliasis;[17] (4) cases of lupus erythematosus with extreme sensitivity to light;[17] (5) some cases of kraurosis vulvae.[17]

Gross[18] reports improvement in cases of kraurosis vulvae by combined administration of vitamin A, riboflavin, and liver extract. He stresses the interrelationship between hormone and vitamin therapy because some of these cases may respond to either type of treatment. He believes some of the very apparent increased effectiveness of crude liver extract may be due to biotin, although it is supposed to be left in the residue of extraction. Two other important observations are made by Gross: (1) pantothenic acid therapy response depends on much larger dosage than is found in vitamin B complex; and (2) very large dosage of thiamine chloride may cause a relative deficiency of nicotinic acid due to increased requirement, although there is no known toxicity to vitamin B complex even in huge doses.

One of us (M. B. S.), in discussing vitamins A and C,[1] mentions certain instances of quicker improvement in phrynoderma (dry skins and follicular keratoses) being effected by *vitamin C* therapy than by administration of vitamin A. He also describes Sjögren's[19] syndrome, which is very similar to the Plummer-Vinson disease in all its manifestations. He states that this condition, which is usually seen in women at the climacteric, is uninfluenced by iron or vitamin B complex, but is much improved by large doses of vitamin A. He also makes a point of the possible relationship between malignant epithelial changes and dysvitaminosis A.

The dermatologic aspects of vitamin C are discussed by Saunders,[20] who states that it exerts a regulatory action on pigment production in scurvy and in Addison's disease, and favorably influences chloasma uterinum. Subclinical scurvy is important in dermatologic diagnosis and management because it is

more common than is usually supposed; and because it may intensify the hemorrhagic tendency of any existing eruption.[17]

Scarborough[21] believes the hemorrhage of vitamin C deficiency to be ecchymotic or deep in muscles and tissues in contrast to that of *vitamin P* deficiency, which is petechial and circumpilar. Gorrie[22] has successfully treated purpura hemorrhagica following neoarsphenamine with vitamin P (hesperidin). Three cases of Henoch-Schönlein type, one of rheumatic and one of symptomatic purpura, were "cured" by Hiramatsu[23] with hesperidin. He believes, however, the effects of vitamin P therapy are enhanced by vitamin C.

At one time or another *vitamin D* has been reported as useful, if not specific, in almost every chronic dermatologic condition. Wise and one of us (M. B. S.) have stated that their experience with vitamin D has been disappointing in the major uses for which it has been recommended in dermatology; namely, psoriasis, acne vulgaris, and pemphigus.[24]

The beneficial effect of *vitamin E* (α-tocopherol) on amyotrophic lateral sclerosis, reported by Wechsler, led Baer[26] to treat a case of *dermatomyositis* with this drug. The patient improved under this therapy, suffered a remission when treatment was stopped, and continued to improve with resumption of α-tocopherol intramuscularly.

The role of the *unsaturated fatty acids* as vitamins is not clear. It is known that certain of these acids, such as linolenic acid, will cure rat "acrodynia" produced by pyridoxine deficiency.[14] Linolenic acid also exerts a vitamin A-like effect on nail nutrition,[27] although its isomer, α-eleostearic acid, is without effect.

Gross[28] has shown that animals fed vitamin B_6, together with curative doses of essential fatty acids, were entirely protected against vitamin B_6 deficiency, but slowly developed the skin lesions of the *filtrate-factor* deficiency and died from the lack of these factors.

SULFONAMIDES IN DERMATOLOGY

The history of the sulfonamides in dermatology is much like that of their use in general medicine. The statement is almost warranted that sulfonamides have been tried on every dermatologic entity except the frank malignancies, nevi, and atrophies. As in other fields of medicine, sulfonamide therapy may be said in many respects to be still in the experimental stages. There are, of course, certain well-recognized dermatologic indications where one of the sulfonamides may be the therapy of choice, others where there is enough established evidence of possible benefit to justify its use, and then the vast limbo of "shooting arrows in the air" and of the "last resort" cases.

It is certainly not within the scope of this paper to discuss the mode of action of the sulfonamides, but the opinion may be ventured that it is probably an effect on the oxygen metabolism of the germ itself. The reader may be referred to authoritative papers on this subject.[29, 30] The selection of the drug itself offers somewhat of a problem, some authors reporting better results in general with *sulfapyridine* than with *sulfanilamide*.

Sulfathiazole is generally accepted as being the agent of choice in staphylococcic infections and is the drug most used in topical application, either as

powder or as the soluble sodium salt. In our own investigations we have found that sodium sulfathiazole is not a primary irritant to the skin, despite the high alkalinity (pH. 11:0). As a preliminary precaution patch tests were applied on 35 normal skins before we used sodium sulfathiazole therapeutically as wet compresses and irrigations. We have used this salt in 5 per cent concentrations in ointments, in emulsions, and as compresses, employing it in infected hemostatic (varicose) ulcers, in impetigo, in furuncles, and in carbuncles. In the latter we have used a complementary oral dosage of 4.0 to 7.0 Gm. daily, trying to maintain a blood level in excess of 2.5 mg. per cent. We can recommend this combination of topical and internal therapy for certain severe pyodermas that do not respond to the usual older measures.

In agreement with observers in other fields of medicine, we have found that, in general, it may be stated that the more acute the dermatosis, the better the results with sulfonamide therapy. Either *sulfanilamide* or *sulfapyridine* has been reported to be definitely useful in the following dermatologic conditions, among others: (1) streptococcic cellulitis and lymphangitis; (2) streptococcic ulcers; (3) erysipelas; (4) erythema multiforme (some types); (5) chancroid; (6) lymphogranuloma venereum; (7) subacute discoid type of lupus erythematosus. There have been many interesting reports of further uses of sulfonamides in isolated cases or in too short a series to be conclusive evidence for general acceptance. Anderson[31] and Abramowitz[32] report cures of patients with acute disseminated lupus erythematosus. Glyn-Hughes and Spence[33] report a series of 12 patients with lupus erythematosus in whom all gave favorable responses and six were cured with sulfapyridine. On the other hand, Wile and Holman[34] report discouraging results with sulfonamides in acute disseminated lupus erythematosus.

Dobson[35] believes sulfanilamide a valuable adjunct in the treatment of actinomycosis. Schoch and Shelmire[36] report success with sulfanilamide in the treatment of erysipeloid of Rosenbach.

Good initial results in dermatitis herpetiformis and sycosis vulgaris have seldom been maintained.

It is almost an impertinence here to discuss the general systemic signs and symptoms of the toxicity of the sulfonamides, since they have been so widely described and so accurately detailed. The cutaneous reactions usually seen are the well-known morbilliform eruptions and the less known dermatitis from photosensitivity, *erythema multiforme-like* and *erythema nodosum-like* dermatoses, scarlatiniform eruptions, fixed eruptions, stomatitis, urticaria, purpura, varioliform eruptions, and exfoliative dermatitis.[37] Sulfathiazole in many instances presents a conjunctivitis or "pink eye" which may be the initial—or even the only—manifestation of intolerance. Baer states he has observed generalization in two patients with psoriasis following a sulfonamide eruption.[38] He believes this to be due to a Koebner phenomenon at the site of local injury due to the eruption. This observation is well to keep in mind with reference to other photosensitizing agents that may be ingested by psoriatics undergoing ultraviolet light treatments.

HORMONAL THERAPY (ENDOCRINOLOGIC THERAPY)

The present enthusiasm for endocrine therapy is on a par with that for the sulfonamides and vitamins. Many liters of *estrogenic substances* and of *anterior-pituitary-like substances* are being administered to women presenting various dermatoses at the menopause. However, specific treatment with *estrogens* in dermatology is almost limited to *senile pruritus* and *kraurosis in the female.* As early as 1935 Davis[39] reported good results with suppositories of 75 R.U. of amniotin (Squibb) in kraurosis. Recently stilbesterol has been reported to be highly effective when given by mouth in doses ranging from 0.1 to 1.0 mg. daily; or, topically, in ointments containing 10 to 20 mg. per ounce.[40] (In the way of non-endocrinologic treatment Saville[41] claims excellent results in both simple leucoplakia and kraurosis with diathermy, using a belt about the hips or a pubic plate and an all-metal electrode high in the vagina. Relief is obtained in four to fifteen treatments, using the factors fifteen minutes and 0.7 to 1.5 amperes.) Lane[42] reports the clearing of an *oral leucoplakia* in two patients by use of *estradiol.*

Folliculin has been advised in *alopecia* for both men and women. *Testosterone* is known to grow hair, sometimes on face and chest, to the embarrassment of young women. We have recently seen several cases of young women treated with testosterone for menorrhagia who presented new growth of hair on face and body, deepening of voice, and a deep pustular acneform eruption, most marked on borders of the face. Moreover, in patients with a tendency to acne, or even in those with only mild seborrheic habitus, many of the androgenic hormones will quite regularly produce acne. The same remark applies to eunuchs or eunuchoid persons receiving androgens.[43] Many of the androgen acnes we have observed have cleared up promptly upon withdrawal of the hormonal therapy (in a few days to weeks). However, a few cases have *persisted for many months despite the stopping of the hormonal administration and the usual anti-acne therapy.*

Feldman[44] states that six patients with generalized senile pruritus were cured by injections of 5 to 15 mg. testosterone proprionate weekly. There are as yet no reports by others to confirm this interesting and important observation.

Brittleness and friability of nails can occasionally be benefited by *thyroid* therapy. Subclinical thyroid hypoactivity may be manifested by no other clinical signs than mild dryness of the skin and hair, or brittleness of the nails. In our experience a fair number of patients with *acne vulgaris* or with *atopic dermatitis* will respond favorably to thyroid medication even though the basal metabolic rate is within the so-called normal limits.

ANTISYPHILITIC THERAPY

In our opinion the outstanding advance in antisyphilitic treatment is the massive arsenotherapy in early syphilis by some of the newer methods, including the *continuous intravenous drip method.*[45] The idea of Sterilisatio Magna began with Ehrlich, and was carried on in the experiments of Linser[46] and Pollitzer.[47] A modern study, begun by Chargin, Leifer, and Hyman in 1932,[48] is a masterpiece of painstaking basic preliminary work and careful clinical and laboratory observation during the treatment. The conservative conclusions

expressed by the whole group at this time are characteristic of the truly scientific attitude of the workers. The original article[45] is on the "must list" for anyone even remotely interested in syphilis or serology. Much of the data concerning serologic comparisons and interpretations has not as yet been published, and will, undoubtedly, of itself be an important contribution. It is impossible to summarize this work since the original report is, indeed, a summary. Certain conclusions regarding the possible advantages of the massive intracutaneous drip method may, however, be safely stated: (1) More patients who began treatment are cured than with the intermittent treatment (during which so many patients disappear as soon as rendered asymptomatic, only to develop later the well-known serious aftermaths of insufficient therapy). (2) The method, when mapharsen is employed, is as safe, or *almost* as safe, as the intermittent treatment in early syphilis. (3) Nitritoid reactions have been almost proved to be a "speed shock" phenomenon and are not seen in this procedure. (4) Mapharsen, as previously believed, has proved efficacious and less toxic than neoarsphenamine. (5) No major dermatitides were seen with mapharsen. (6) Serious manifestations of toxicity occur with neoarsphenamine—hemorrhagic encephalitis, peripheral neuritis, hepatitis. (7) Relapses do occur. (8) The late results, including both those due to the syphilis itself and to massive dose arsenic therapy, are not yet known. (9) It is, as yet, only an experiment, but nevertheless, an intensely interesting and valuable one.

However, as one of us (M. B. S.) has previously stated: "It is, for example, not yet known whether mapharsen could not be given several times daily by means of small syringes with effects equivalent to those obtained when the same dose is given by continuous drip."[49] At present it appears to us probable that the results obtained with mapharsen in the intravenous drip may be due not so much to the beneficial effects of the *slow drip method,* as to a *previously unrecognized lack of toxicity or broad therapeutic index of mapharsen itself in man.* If this theory proves true, one should be able to give daily large doses of mapharsen in ambulatory patients, with small syringes and without the difficulties and expense of hospitalization and drip administration.

Very recent studies, particularly those of Schoch and Alexander,[50] and collaborators, support the statement just quoted from the Year Book and our present opinion. For these investigators have now treated over 40 patients with early syphilis with *daily doses of 0.12 Gm. of mapharsen.* Each dose was given rapidly in 10 c.c. of water with a small syringe. The patients all were ambulatory, all continued with their occupations; none experienced the slightest untoward effects or untoward reactions. In this as yet small series the results are most promising. The disappearance of the syphilitic manifestations and the reversal of the serologic reactions were fully as good as with the drip method.

A further advance in antisyphilitic therapy is the introduction of the drug *sobisminol,*[51] which can be given orally (as well as by injection) and orally compares favorably in its action with injectable bismuth preparations. However, the bismuth in this form of medication is eliminated rapidly. No depot or constant source of absorption is established with sobisminol,[52] and *clinical relapse* has been reported on its substitution for injectable bismuth. In one

report it has seemed to have an especially beneficial influence in patients with late neurosyphilis of the tabetic type.[53] This benefit apparently regresses with cessation of treatment, but the drug can be given for months without signs of cumulative effects. This does not mean that it is without either major or minor toxic effects of bismuth. In our hands it has been used with gratifying results in two patients with lupus erythematosus, and in one patient with lichen planus. Other authors have reported similar good results.

The discovery by Cole, De Oreo, Driver, Johnson, and Schwartz[54] represents not only a great therapeutic advance but a singularly fascinating subject for further theoretical and practical studies. These observers found that certain bismuth salts, particularly *thiobismol*, control the bouts of therapeutic malaria in the most effective manner. By controlled administration the patient can be kept free of fever and paroxysms whenever desired, and by stopping the drug the paroxysms can be allowed to recur whenever indicated. In other words, the bismuth salt keeps the patient free of malarial attacks but does not destroy the infesting plasmodia, or at least not to such a degree as to eliminate the disease; so that, after the desired interruption, the malarial treatment can be resumed without the necessity of a reinoculation. The theoretic interest as well as the practical advantages of this discovery are both great and obvious.

Saenz, Triana, and Armenteros[55] have discovered in *pinta* patients a *spirochete* morphologically identical to the *S. pallida* and *S. pertenuis*. This disease has long been known to possess most of the serologic characteristics of syphilis and the discovery reawakens or intensifies the interest in the study of cross immunity between syphilis, frambesia, and pinta. It is the ambition of every dermatosyphilologist to find an innocuous disease which will give a cross immunity to syphilis, paralleling that achieved in smallpox with cowpox virus (vaccine). However, little is as yet known about the immunologic relationships or the effects of extracts of microorganisms and of attenuation procedures in various human spirochetoses.

A promising aid in antisyphilitic therapy is embodied in the report by MacKee and Astrachan[56] of the mitigating influence of whole crude liver extract on the erythrodermas incident to antisyphilitic treatment. It was even found that certain individuals could continue receiving cautiously administered arsenical treatment after a major arsenical dermatitis if crude liver extract were given intramuscularly. This report represents a confirmation of, and an addition to, the findings of Genner[57] and others.

We believe the combination of a titered Kolmer, Wassermann, and Kline diagnostic and exclusion tests are sufficient, in almost every instance, for serologic diagnostic purposes and for evaluation of treatment results. The Laughlen test, with the three-minute serum inactivation modification,[58] may be valuable for pretransfusion tests.*

MISCELLANEOUS THERAPY AND PROPHYLAXIS

The recent work on *poison ivy* dermatitis assumes tremendous added importance when considered in connection with the defense program. A majority of

*The use of antigen prepared from spirochetes, as developed by Gaehtgens and reported by Erickson and Eagle (Abstract, Year Book of Dermat. and Syph., p. 733, 1941) may prove a decided advance in diagnostic serologic tests for syphilis.

the military trainees are likely to be "ivy sensitive," and to be exposed this summer. Shelmire[59] has found that the dermatitis-producing excitant is not an oil, but a dialyzable fraction of the oleoresin, soluble in water and in urine. He also reports that the excitant is not carried in smoke, as previously believed. A large and comprehensive study of *oral densensitization* is now being carried out on a nationwide scale, due to Shelmire's experiments in oral prophylaxis, which represent an extension and modification of the older work of Jay Schamberg, Strickler, and others.

Schwartz and Warren[60] have developed and advocated the use of a *sodium perborate-containing cream* for the prevention of poison ivy dermatitis. While these authors submit observations supporting the efficacy of the cream, Shelmire, on the other hand, presents experiments tending to show its lack of effective protection.[61] The value of this cream is, therefore, still awaiting the final judgment of practical results on large series of exposed persons.

The treatment of *chronic ulcers* with *zinc peroxide paste* or *mass* is a valuable and relatively recent therapeutic measure. Rigid attention to selection of the powder—since not all zinc peroxide is active—preparation of the "mass" and of its application must all be observed. An excellent presentation of the rationale and technique is found in the paper of Meleney and Harvey.[62]

We have found application of the *Aloe vera* leaf[63] *alva gel,*[64] and cod allantoin ointment effective in some chronic ulcers, especially the use of the leaf in some of the ulcers following radiodermatitis.

Pliofilm mitts and sheets are useful in retaining wet dressings of all types.[65]

The development of a refined test material (*Lygranum*) for the diagnosis of *lymphogranuloma venereum* (lymphopathica venerea) by the method of Rake, McKee, and Shaffer,[66] promises to be a notable advance. In this procedure the yolk sac of the chick embryo is inoculated, and a practically pure and highly concentrated test material is obtained. We suggest that the Frei test, with original human material, and tests with this new material, be performed in parallel and in a comparative manner in a large series of cases of (1) acute and chronic, suppurative and nonsuppurative inguinal adenitis; (2) acute and chronic proctitis; (3) fistulas of rectum and genitals; (4) acute and chronic ulceration of the genitals; (5) localized elephantiasis.

Semon and Hermann[67] report improvement in *acne vulgaris* in both men and women following the administration of either protamine zinc or ordinary *insulin*. Dosage was usually 5 to 15 U. three times weekly, given without any adjuvant treatment. Exacerbations occurred upon cessation of treatment and improvement began within forty-eight hours of its resumption. The insulin therapy sprang from observations of the lag in sugar tolerance curves in acne patients. The authors state it is impossible, at the present time, to "differentiate between metabolic vagaries and disturbance of hormonal balance."

A recent innovation in the treatment of *intractable pruritus* is the *tattooing* of the anal area with red sulfide of mercury (Hollander, E.[68a] and Turrell, R.[68b]). Good results are reported in the majority of cases.

The use of trichophytin in erysipelas-like infections associated with dermatophytosis of the feet, as advocated by Sulzberger, Rostenberg, Jr., and

Goetze,[69] is worth trying. Individuals in whom a favorable response is expected are those showing an *immediate wheal reaction* to intracutaneous test. The dose is 0.1 c.c. of the usual 1:30 dilution given intracutaneously at five- to seven-day intervals.

The work of Becker[70] and others with *lipocaic* (Dragstet and co-workers), although apparently productive of no lasting results in *psoriasis,* is extremely interesting. The substance is a neutral, insulin-free and fat-free alcoholic extract of pancreas which apparently has selective power of converting some fats to glucose.

Conrad, Conrad, Mapother, and Weiss[71] report the use of bismarsen as a valuable addition to the therapy of lichen planus; 0.1 Gm. given intramuscularly twice weekly exerted a favorable influence on the ordinarily refractory lesions of the mouth, of the penis, and of the hypertrophic type. Vitamin C should be administered while the bismarsen is being given.

In closing, it may be appropriate to give some direct advice from Becker:[72] "Therapy consists, first of all, in removing precipitating factors, social and environmental; secondly, in relieving the underlying instability and exhaustion; and thirdly, in application of orthodox measures."

The principles enumerated are not only good but are long established. However, there is much room for discussion regarding the order in which Becker here mentions these principles. If the order of mention is to be that of relative importance, this will depend not only upon the training, viewpoint, and inclination of the physician, but also upon the particular constellation of circumstances bearing upon the particular case at the particular moment.

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CLINICAL AND EXPERIMENTAL

THE EFFECT OF THE SUBCUTANEOUS ADMINISTRATION OF PROTAMINE (SALMINE) TO RABBITS AND MICE*

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FOLLOWING the announcement of Hagedorn[1] in 1935 that the addition of protamine greatly prolongs the action of insulin, this substance has been used widely as a constituent of protamine insulin and protamine zinc insulin. If used in small doses, protamine apparently has no deleterious effect upon the organism. However, since over a period of years many diabetic patients take relatively large quantities of insulin, the question has arisen as to how large amounts of protamine insulin can be given safely.

Forty years ago Thompson,[2] working in Kossel's[3] laboratory, reported the results obtained when protamine was injected intravenously into narcotized dogs. He found that a dose of clupeine (originally regarded as chemically identical with salmine) greater than 15 to 18 mg. per kilogram could not be given without causing death of the animal. Following the injection of protamine a definite and relatively prompt fall of the blood pressure took place. After a nonfatal dose, the blood pressure regained the normal level in twenty-five to thirty minutes. Simultaneous with the fall in the blood pressure after a nonfatal dose, the respirations at first became increased in rate and depth; then followed a period of rest and finally gradual resumption of normal breathing. It was noteworthy, however, that active thoracic breathing was absent as long as the effect of the protamine lasted. In addition to the effects upon the blood pressure and the respiration, Thompson noted delayed clotting of the blood and a diminution in the number of circulating leucocytes.

Jappelli[4] in 1933 repeated Thompson's experiments in unanesthetized animals. Doses of salmine of 20 mg. per kilogram of animal caused agitation, dyspnea, salivation, diarrhea, and a drop in the arterial blood pressure. A second dose caused a further drop in arterial pressure, and a third was fatal (vasomotor paralysis).

According to Walther and Ammon,[5] three guinea pigs of 250 to 300 Gm. body weight survived two intraperitoneal injections each of 5 to 10 mg. of protamine. They died, however, following intracardial injections of 35 mg. four weeks later.

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Because in the literature there are no data regarding the toxicity of protamine when given subcutaneously, we have determined the lethal dose for mice and rabbits and have studied the signs and symptoms which follow the administration of large amounts.

RESULTS

Lethal Doses.—The lethal dose following subcutaneous injection was determined in 40 mice and 23 rabbits.

Female mice weighing from 12.0 to 33.5 Gm. were used. Food and water were withheld during the first twelve hours after the administration of protamine, but were allowed before and after that time. The neutralized protamine* was given subcutaneously in 1 per cent concentration in a physiologic solution of sodium chloride. The behavior of the animals was observed for five days after the injections.

As may be seen in Table I, the average or median lethal dose of protamine in our experiments was about 250 mg. per kilogram of animal. All deaths occurred within one hour after injection.

TABLE I

MORTALITY OF MICE AFTER A SINGLE SUBCUTANEOUS INJECTION OF PROTAMINE (SALMINE)

DOSE PER KG. ANIMAL (MG.)	NUMBER OF MICE TESTED	NUMBER OF MICE DYING	TIME OF DEATH AFTER INJECTION (MIN.)
400	2	2	18-32
300	10	8	19-55
250	10	5	23-60
200	10	1	40
100	4	0	
50	2	0	
25	2	0	

TABLE II

MORTALITY OF RABBITS AFTER A SINGLE SUBCUTANEOUS INJECTION OF PROTAMINE (SALMINE)

DOSE PER KG. ANIMAL (MG.)	NUMBER OF RABBITS TESTED	NUMBER OF RABBITS DYING	TIME OF DEATH AFTER INJECTION (HR.)
500	2	2	4-10
300	8	3	8-16
200	5	3	5-12
100	4	0	
50	2	0	
25	2	0	

Male rabbits weighing 1,180 to 2,760 Gm. and fasted twenty-four hours were used. No food or water was allowed during the first twelve hours after injection of protamine. The behavior of the animals was observed during the following five days. The protamine was injected in 10 per cent concentration in a physiologic solution of sodium chloride. Before injection these preparations were warmed to about body temperature in order to diminish the turbidity which was present in the cold.

*The protamine used was salmine (Lot No. 961271) prepared by Eli Lilly & Co., Indianapolis, and furnished to us without cost.

The median lethal dose of salmine injected subcutaneously seemed to be essentially the same in rabbits as in mice (Table II), being between 200 and 300 mg. per kilogram of animal (a dose of 250 mg. per kilogram was not used with the rabbits). However, the results were not as uniform as in the mouse experiments; it is possible that this may have depended upon the more delayed and uneven absorption of protamine when injected in 10 per cent, rather than 1 per cent, concentration. In contrast with the experience with mice, only one of the rabbits died during the first four hours after an injection.

Signs and Symptoms Following the Subcutaneous Injection of Large Amounts of Protamine.—One of the most striking early signs noted following the subcutaneous injection of salmine was the dilatation of superficial blood vessels, as seen particularly in the ears of rabbits. This was first observed usually fifteen to twenty minutes after an injection and reached its maximal intensity thirty to ninety minutes after the injection. Although barely demonstrable after the smaller doses used (50 to 100 mg. per kilogram), it was marked after the larger doses. The large ears of white New Zealand rabbits became warm and strikingly bluish red.

Beginning with the third hour after injection, there appeared in some animals certain signs of blood vessel damage, especially after doses larger than 200 mg. per kilogram. Thus pressure upon the ear with the finger tips produced blanching which persisted for an unusually long time. The prominence of this feature depended somewhat upon the degree of dilatation of the superficial blood vessels at the time pressure was applied. In a few cases there was to be seen at about the fourth hour following injection a marked edema of the ears which remained visible the following day.

In most of the fatal cases the rabbits had marked respiratory distress, beginning one to two hours after the injection. Breathing became very labored and slower than normal. Sometimes râles could be heard, suggesting pulmonary edema. At this stage the animals often assumed a position of opisthotonos, with the nose lifted up and the ears directed along the back. At times this position was maintained by supporting the chin on a base, as on the edge of a basket or pan. After doses of 100 to 500 mg. per kilogram there sometimes occurred rigidity of the animal, which reached a maximum in the fifth to seventh hour after injection, and occasionally could still be seen the following day.

Following doses of from 50 mg. per kilogram and more, the animals seemed to suffer from marked malaise between the first to fifth hours and after large doses this persisted even longer.

Between the second and sixth hours many rabbits showed a definite increase in the output of urine; this was usually very light and clear. The urine occasionally reduced Benedict's solution but to no greater extent than did 0.15 per cent glucose. During a period in which rabbit P16 (dose of protamine, 200 mg. per kilogram) had marked hyperglycemia, no urine was obtained for sugar determinations. Rabbits P22 and P25 (dose of protamine, 300 and 200 mg. per kilogram, respectively), showing marked changes in the kidneys at post mortem, had in the urine albumin, granular casts, red blood corpuscles, white blood cells, and epithelial cells.

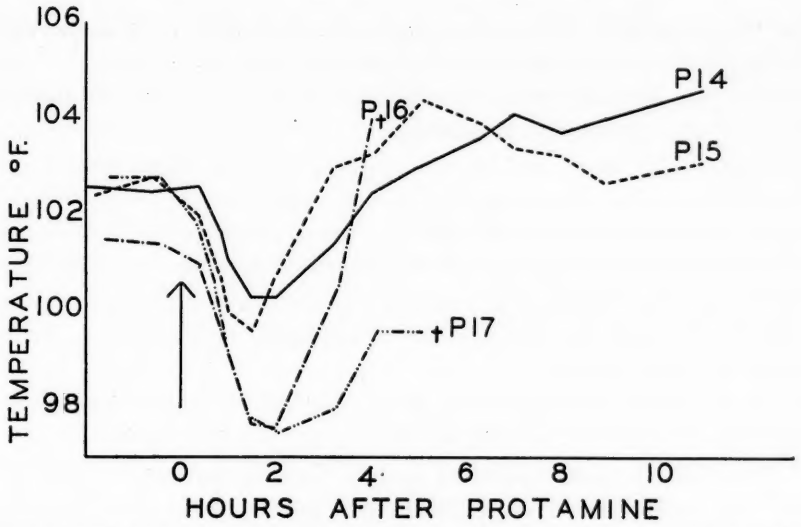

Chart 1.—Effect of the subcutaneous injection of protamine on the rectal temperature of rabbits. Animals P14 and P15 received 100 mg., and P16 and P17 200 mg., per kilogram of body weight. The sign + indicates death of the animal.

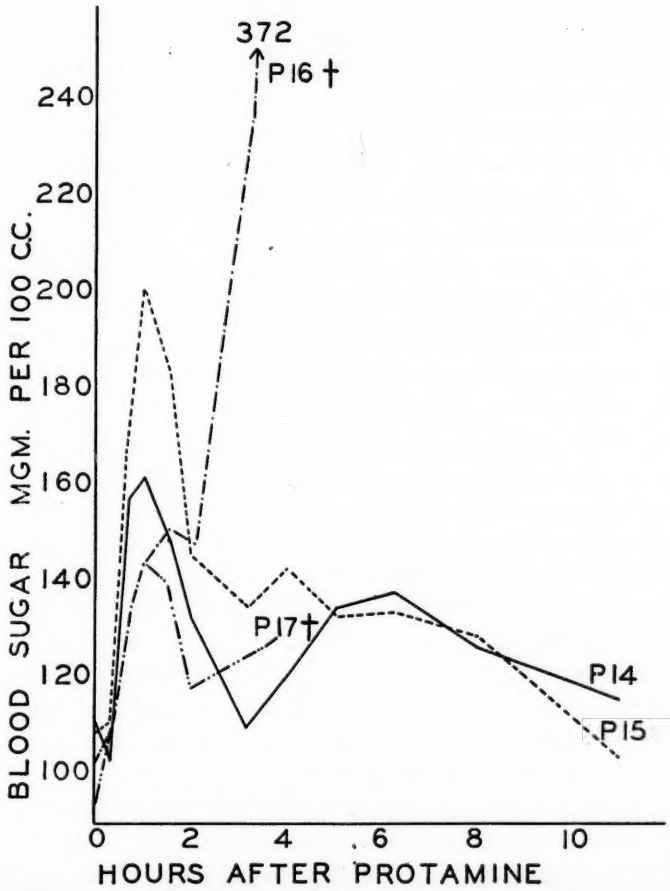

Chart 2.—Effect of the subcutaneous injection of protamine on the blood sugar of rabbits. Animals P14 and P15 received 100 mg., and P16 and P17 200 mg., per kilogram of body weight. The sign + indicates death of the animal.

One of the rabbits (P25) developed, after the fifth hour following injection, paralysis of left anterior and right posterior limbs.

Some of the animals showed between the first and third hour the frequent defecation of a moist mucous fecal mass.

In a group of eight rabbits the changes in body temperature after the injection of protamine were studied by hourly rectal measurements. The largest doses used in these experiments, 200 mg. per kilogram, caused a marked fall of body temperature within two hours after the injection of protamine. After that, the temperature rose again, reaching levels above normal. Even the smaller doses (25 to 100 mg. per kilogram) had a similar effect, that of 25 mg. per kilogram being very slight.

The blood sugar, studied in the same eight rabbits, showed marked alterations (samples of 0.1 c.c. were taken from a marginal ear vein, and the sugar content was determined according to the method of Folin and Malmros[6]). During the first two hours the values were increased, showing a maximum usually at one hour after the injection of protamine. During a second rise, which began usually in the third hour, one of the animals (P16) had premortal values as high as 372 mg. per 100 c.c.

The symptoms in mice after injections of protamine corresponded to those in rabbits, although they were more difficult to follow accurately. After a dose of 200 to 400 mg. per kilogram of animal, all the surviving mice seemed very ill during the first one to five hours. Six animals were opisthotonic about ten to fifty minutes after the injection. Many mice had marked rigidity of the body about one hour after the injection. Some of the animals had clonic convulsions a short time before death; others died with the body flaccid.

After a dose of 50 to 100 mg. per kilogram of animal, the mice seemed to suffer malaise for a few hours. The smallest dose of 25 mg. per kilogram seemed to have no effect upon the animals.

*Post-mortem Findings.**—The most striking and constant finding on examination of the *rabbits* which died were hemorrhages in the thymus gland. These varied in extent from areas of pin-point size to massive involvement of the gland. The extent of the hemorrhages did not seem to depend absolutely upon the size of the dose of protamine used, although all animals dying had received relatively large amounts. The lungs showed many pleural and parenchymatous hemorrhages of varying size, and frequently there was bloody foam in the trachea and bronchi. The changes in the lungs seemed to parallel roughly in extent those in the thymus. The kidneys showed cortical and subcortical foci of hemorrhage and necrosis, suggesting infarcts, from pin-point size to 5 mm. In *mice* the most constant findings were in the lungs, which were edematous and often showed tiny points of hemorrhage on the surface. Hemorrhages were noted also on the surface of the thymus and adrenal glands.

Microscopic study of the tissues confirmed the findings made on gross examination. In addition, the liver showed some degree of focal necrosis with polymorphonuclear infiltration; in one instance the process was widespread with

*The necropsies reported in this paper were carried out immediately after the spontaneous death of the animals. Tissues were placed in formalin or Zenker's fluid.

involvement of about half of the liver tissue in the sections examined. Degeneration of the tubular epithelium of the kidney with many hyaline casts in the tubules was seen. In one rabbit a small area of focal necrosis with polymorphonuclear infiltration was seen in one adrenal gland.

DISCUSSION

The data presented in this paper demonstrate that protamine (salmine) is definitely toxic when given subcutaneously in large amounts. However, the doses required for the appearance of abnormal signs are relatively enormous.

A dose of protamine zinc insulin of 100 units is from two to three times as great as that used daily by a patient with diabetes of moderate severity. According to present standards, 1.25 mg. of protamine are contained in each 100 units of protamine zinc insulin. For a patient weighing 65 kg. this would represent roughly 0.02 mg. per kilogram, or about $\frac{1}{10,000}$ the minimal lethal dose as established in our work for mice and rabbits. Furthermore, the tendency to toxic effect probably is lessened because of the tolerance gradually set up by the daily subcutaneous administration. At any rate, in an extensive use of protamine-containing insulin in hundreds of cases of diabetes treated since 1935 at the George F. Baker Clinic, no harmful effects have been observed which could be attributed to the protamine per se. Local responses consisting of redness, swelling, heat, soreness, and itching at the site of injection are frequently seen, but even these effects usually become less and disappear after injections have been continued for several days or at the most, a few weeks.

Although we have regarded these local responses to protamine zinc insulin as allergic in nature, Kern and Langner[7] doubt this because they obtained negative intracutaneous reactions with a protamine solution in 104 diabetic patients treated with protamine zinc insulin. They quote Taylor[8] and Wells[9] to the effect that protamines have no antigenic properties; furthermore, they were unable to induce sensitivity to protamine in four guinea pigs.

In an article already mentioned, Walther and Ammon[5] interpret the death of three guinea pigs, each of which was given two parenteral injections of protamine four weeks apart, as due to anaphylactic shock. It is possible that the fatalities observed by Walther and Ammon occurred on a purely toxic, rather than an allergic, basis. Walther and Ammon gave as their second or "shocking" dose, 35 mg. of protamine to a guinea pig weighing 250 to 300 mg., or approximately 115 to 140 mg. per kilogram. Although this is somewhat below the median lethal dose which we found in mice and rabbits after subcutaneous administration, nevertheless, it is a large dose and may have been more effective, given as it was, intracardially.

The events which are observed to follow the injection of large amounts of protamine into rabbits and mice, together with the pathologic findings, probably are not specific, resembling in many respects those seen after the parenteral introduction of various foreign substances, including proteins. To some extent our findings resemble those observed by Selye[10] following sublethal doses of various drugs, as histamine, adrenalin, atropine, morphine, and formaldehyde. The "alarm reaction" produced is considered by Selye to represent simply the response of the organism to a damaging stimulus.

SUMMARY

1. The effect of the subcutaneous administration of large amounts of protamine (salmine) to 40 mice and 23 rabbits was studied.

2. The median lethal dose was found to be about 200 to 300 mg. per kilogram body weight.

3. The symptoms and signs produced by the injection of protamine consisted of respiratory distress, muscular rigidity, and opisthotonos, marked malaise, polyuria, fall in body temperature followed by a rise, and hyperglycemia. Dilatation of superficial blood vessels with some evidence of damage was noted.

4. Post-mortem examination of animals dying as the result of an injection of protamine showed signs of vascular damage with hemorrhages into the thymus, lungs, and kidneys. Other findings included infarcts in the kidneys, acute toxic changes in the kidney tubules, and focal necrosis of the liver.

5. It is concluded that the amount of protamine contained in insulin used in the treatment of diabetes represents so small a fraction of that found harmful to rabbits and mice that the possibility of its being a hazard is remote.

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PRELIMINARY OBSERVATIONS ON LOWERING OF BODY TEMPERATURES*

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"PROFOUND oblivion midway between sleep and death" may be poetic eloquence, yet it expresses the intermediate stages of lethargy resulting from the associated efforts of sedation and refrigeration. The lowering of body temperature to approximately 20° F. in the dog by artificial means simulates natural hibernation in the upper strata of mammals.

The following report will endeavor to picture the physiological events occurring with metabolism at its apparently lowest ebb, and yet this ebb is compatible with life. To achieve this end, which others have suggested to be a palliative consideration for generalized cancer disease, a technique revolving around the simple idea of electrical refrigeration was employed.

In order to produce this lowered body temperature in a controlled manner, a cabinet cooled by electrical refrigeration was devised. This consisted of a rectangular wooden box insulated with several layers of cork. A swinging door, likewise insulated, was arranged to allow for free access to the interior, which measured 24 by 12 by 12 inches. The cabinet lay on its long side, facilitating such matters as laying the animal in a comfortable position and placing the necessary recording instruments. A metal shelf upon which the dog rested was set 6 inches from the floor of the cabinet, thus assuring sufficient byways and highways for the proper physics of refrigeration. From the ceiling of the box were suspended coils connected to the compressor, thereby completing the refrigerating system. In the ceiling and the floor were two one-inch vents, solving the problem of aeration for the animal. Emerging from the top of the cabinet, closely situated to the corners, were outlets just large enough to permit the exits of a ¼ inch rubber tube for the intravenous anesthetic, a capillary tube from the rectal thermometer, a tube from the cabinet thermometer, a tube from the interior to the humidistat, a tube from the pneumograph to its manometer, a tube from the femoral artery to its pulsometer, and two metal tubes from the coils to the compressor unit.

Behind, and on the same stand with the cabinet, was placed the ¼ H. P. compressor unit capable of 2,000 B.T.U.'s. Difluorodichloromethane was used for the refrigerant. This unit was capable of lowering the temperature of the interior of the cabinet (no animal) from 69° F. (room temperature) to 32° F. within eighty minutes.

*From the Mattoon Polyclinic, Mattoon.
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Anesthesia of the dogs was produced and maintained by continuous intravenous gravity (drop) method with a 0.5 per cent solution of pentothal sodium. This was given in all cases.

The dogs used were very much alike in type, weighing about 15 pounds. For obvious reasons, they were chosen for their short hair.

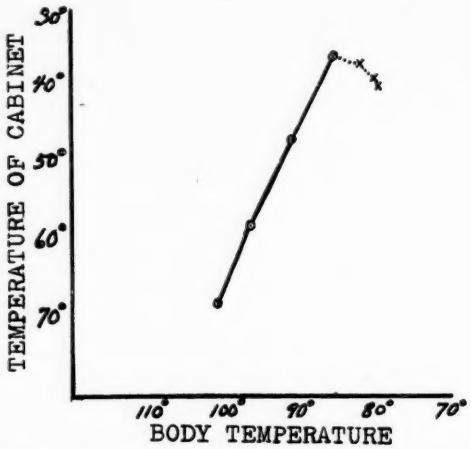

Chart 1.—Body temperature and cabinet temperature.

The actual experiment was conducted in the following manner: First, a ½ inch No. 18 needle was inserted into the right cephalic vein and connected to the adapter of the intravenous apparatus. Sixty drops per minute induced the animal within ten minutes to the stage of insensibility. The dog was then placed upon the metal shelf so that it lay on its right side with its feet pointing outward. The pneumograph was connected around the lowest part of the chest. The pulsograph was taped to the left femoral artery. The thermometer was inserted deeply into the rectum, the tail was allowed to drop down between its legs and fastened in this fashion to secure a permanent position of the rectal thermometer. Everything was in readiness for the actual cooling after a normal set of readings of the pulse, respiration, temperature of the animal, temperature of the room, and the humidity was recorded.

Fifteen dogs were submitted to refrigeration under anesthesia. Two died in the early experiments due to an overdosage of the pentothal sodium. To study the effect of insulin and metrazol on the lowered body temperature, two other animals were sacrificed. Both injections were made when the body temperatures were below 80° F. The observations made thereof were extracurricular, and consequently, were not considered in this report. The remaining 11 dogs passed through the experiments and apparently returned to a normal condition. Two days after completion of the experiments they were observed to be in a normal state of health.

The observations considered in this report are based on the average of all the findings in these experiments, since variations from the average are negligible. For the sake of consistency, the same assistants recorded all the data. No untoward symptoms were encountered. No complications arose.

After the cooling apparatus was put into operation, readings were taken every fifteen minutes, however, now supplemented by the cabinet temperature.

Chart 1 contains changes of body temperature as compared with the cabinet temperature. From an apparent normal body temperature of 100.8° F., the temperature dropped continuously with refrigeration. The lowest recorded body temperature was 79.5° F. whereas, at the same time, the cabinet temperature was 44° F. It was interesting to note that the cabinet temperature had to drop to 32° F. to make the "holdover" to carry on the lowering process. At this point the compressor was stopped, and as was expected, the body temperature gradually rose along with the cabinet temperature.

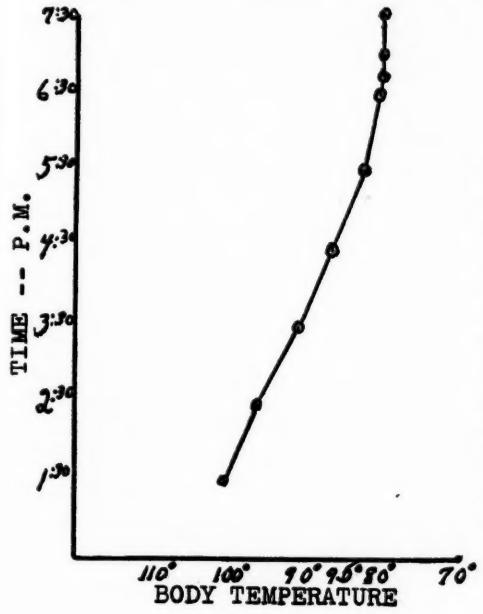

Chart 2.

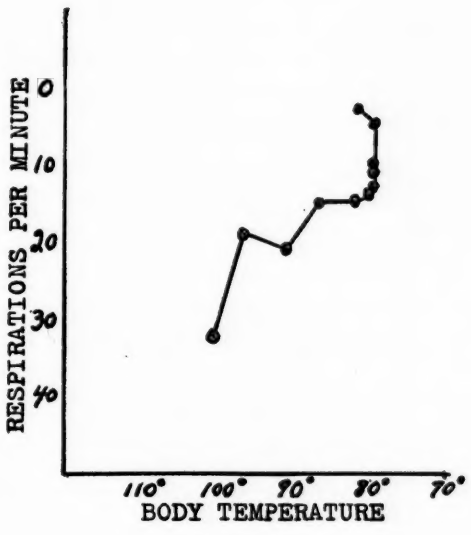

Chart 3.

Chart 2.—Time for body temperature to reach lowest point.
Chart 3.—Respiration and body temperature.

Chart 2 elaborates the changes of body temperature as compared with the time required to make these changes. The ¼ H.P. compressor unit required six hours to lower the body temperature from normal to 79.5° F. This time element is striking as compared to the more than eleven hours necessitated for the body temperature to return to normal.

Chart 3 illustrates that respiration which decreased with the lowering of the body temperature was a more sensitive physiologic sign for judging the dog's condition than the pulse. Unlike Charts 1 and 2, Chart 3 is marked by the absence of a "straight" curve by being spotted with a number of fluctuations. The significance lies in the fact that respirations decreased to three per minute with the body temperature at 79.5° F.

Chart 4 compares the pulse changes with those of the body temperature. The former usually dropped along with the latter. When the body temperature registered 79.5° F., the number of recorded pulsations per minute was 60.

However, the changes of the pulse rate did not occur as acutely as did the respiratory rate.

Chart 5, probably not as significant as the other charts, demonstrates the changes in humidity of the cabinet as related to the body temperature. The humidity rose as the body temperature fell.

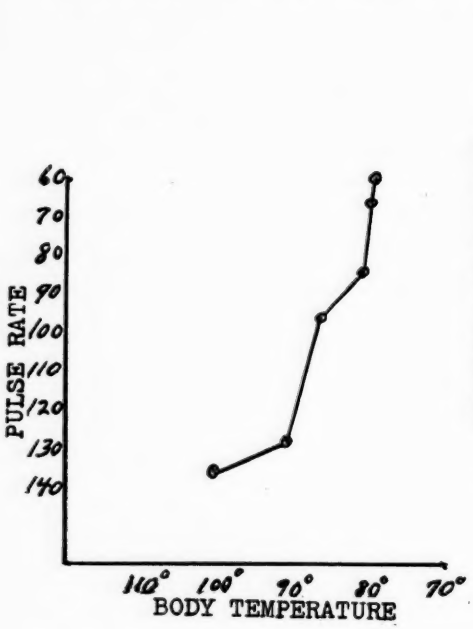

Chart 4.

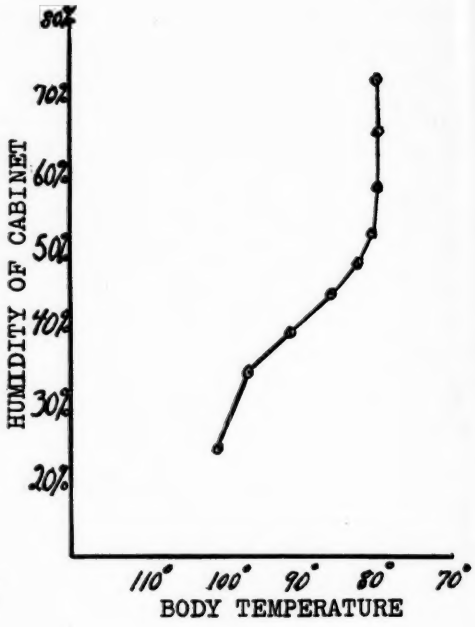

Chart 5.

Chart 4.—Pulse rate and body temperature.
Chart 5.—Humidity of cabinet and body temperature.

SUMMARY

The convenient method of lowering body temperature of dogs by the use of an electrical refrigeration unit has been described. The physiologic changes observed are:

1. The body temperature dropped to 79.5° F. when the interior cabinet temperature was 44° F.

2. The time required for the above was six hours, almost half the time it took the dog to return to a normal temperature.

3. The respiration dropped to three per minute when the rectal temperature read 79.5° F. The respiratory rate was a more reliable indication of the condition of the dog than was the pulse rate.

4. The pulse rate dropped to 60 per minute as the body temperature dropped to 79.5° F.

5. The humidity of the interior of the cabinet was 72 per cent with the body temperature at its lowest, 79.5° F. This reading became lower as the body temperature increased. The humidity may have been significant as a prophylaxis for pneumonia more than for its physiologic importance.

Detailed deductions have been withheld pending completion of experiments on diseased dogs.

ALLERGIC PNEUMONIA*

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THE importance of the lung as a shock organ in allergic disease is now a commonplace. That true bronchial asthma is a manifestation due to the reaction of hypersensitiveness is universally accepted. In this condition the allergic reaction is confined to the bronchial tree.

A consideration of allergic pneumonia necessitates an entirely different concept. That an allergic response, including the *interstitial pulmonary* tissues, possibly is involved in the production of typical lobar pneumonia (pneumococcal) as well as certain types of pulmonary tuberculosis has long been considered.

However, an extensive pneumonitis on the basis of allergic response to agents other than those of infection is a relatively new and somewhat startling concept; namely, that the interstitial tissue of the lung is capable of sensitization and of reaction to foreign substances. That this may possibly be the case, however, is suggested by numerous reports. Loeffler[1] in 1932 described a disease to which he gave the name, "Flüchtige Succedan-Infiltrate (mit Eosinophilie)." By 1936 Loeffler[2] had collected a total of 51 cases, characterized by infiltrative processes in the lung (demonstrated by x-ray), of leucocytosis and eosinophilia. In many instances other symptoms were few or lacking entirely, such cases being discovered through the procedure of group examination for tuberculosis. Reports of a similar condition include those of Busche,[3] Wild,[4] Engle,[5] and Leitner.[6] Gravesen[7] in 1938 reported a case strikingly similar to the one presented in the following report, and suggested an allergic etiology. His patient, like the following, was receiving prontosil medication.

CASE REPORT

History.—S. L., a white male, aged 22 years, a law student at the University of Minnesota, admitted to the hospital January 15, 1939, complained of (1) frequent loose stools, (2) abdominal tenderness, (3) headache, (4) chills and sweats. The history of previous illness was unimportant except in relationship to the present complaints. In October, 1936, while he was in another city, he had a sudden onset of diarrhea, his stools containing bright red blood and mucus, for which he was hospitalized about six weeks. He returned to school in January, 1937. He had occasional recurrences of loose stools, but none were severe prior to the episode responsible for this admission.

Examination.—His temperature on admission was 100.6° F., pulse rate 106, respiration 22. Nutrition was fair. There was moderate abdominal distention with left lower quadrant tenderness. Physical examination otherwise showed nothing remarkable. Proctoscopic examination

*From the Students' Health Service and the Department of Medicine, University of Minnesota.

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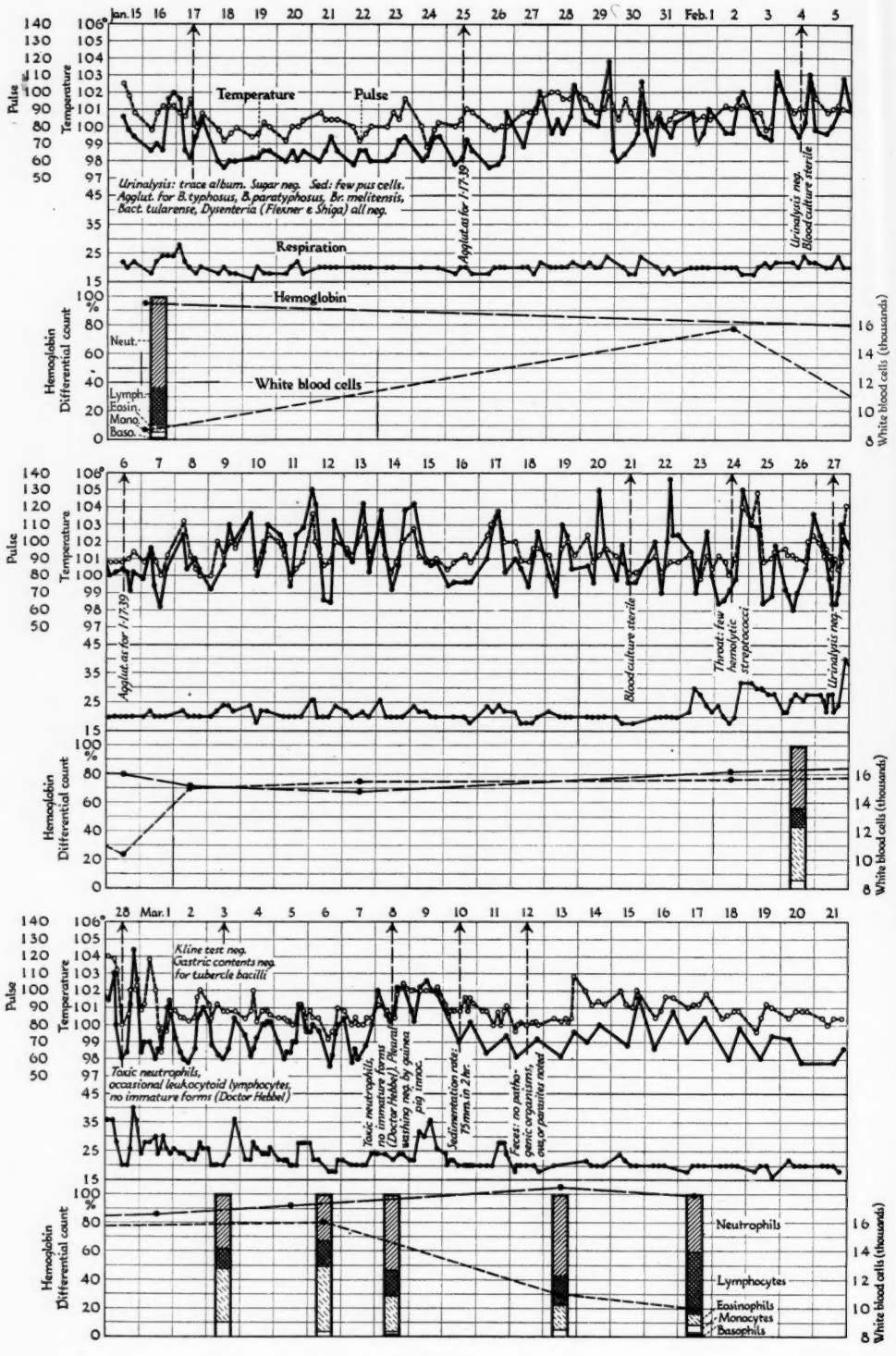
Jan. 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 Feb. 1 2 3 4 5
Pulse
Temperature
Temperature
Pulse
Urinalysis: trace album. Sugar neg. Sed: few pus cells. Agglut. for B. typhosus, B. paratyphosus, Br. melitensis, Bact. tularense, Dysenteria (Flexner e Shiga) all neg.
Agglut. as for 1-17-39
Urinalysis neg. Blood culture sterile
Respiration
Hemoglobin
White blood cells
Hemoglobin Differential count
Neut.
Lymph.
Eosin.
Mono.
Baso.
White blood cells (thousands)
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Agglut. as for 1-17-39
Blood culture sterile
Throat: few hemolytic streptococci
Urinalysis neg.
28 Mar. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Kline test neg. Gastric contents neg. for tubercle bacilli
Toxic neutrophils, occasional leukocytoid lymphocytes, no immature forms (Doctor Hebbel)
Toxic neutrophils, no immature forms (Doctor Hebbel). Pleural washing neg. by guinea pig inoc.
Sedimentation rate 15mm. in 2 hr.
Feces: no pathogenic organisms, ova, or parasites noted
Neutrophils
Lymphocytes
Eosinophils
Monocytes
Basophils

Chart 1.

by Dr. H. W. Christianson showed the bowel mucosa studded with pin-point ulcers which bled easily. Treatment prescribed by the proctologist included prontosil by mouth and a streptococcus vaccine parenterally. Prontosil was administered in 15 gr. doses every six hours (60 gr. daily) from January 25 to March 6, inclusive.

Clinical Course.—The illness with which this report is primarily concerned is considered to have started with a recrudescence of fever without other obvious new developments, and at a time when the colitis was showing marked improvement. The temperature was moderately elevated on admission but became normal two days later. During the succeeding week slight elevations of temperature occurred not exceeding 99.4° F. On January 25, the tenth day following admission, prontosil medication was initiated, with two 15 gr. doses at 6 P.M. and 10 P.M., respectively. On succeeding days the patient received four 15 gr. doses at six-hour intervals. On the evening of the eleventh hospital day (first full day of prontosil medication) the temperature rose to 100.8° F., the following day to 102° F., and on the next day to 103.8° F. Chilly sensations were mentioned, and nonproductive cough developed. No abnormal signs were noted on repeated physical examinations. X-ray of the chest on January 31 (seventeenth hospital day) showed marked thickening of the pleura, especially over the left apex, and diffuse increase in the bronchovascular markings, especially in the left lower lobe (Fig. 1).

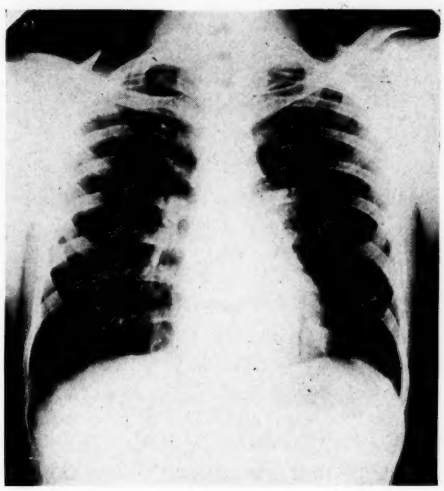

Fig. 1.

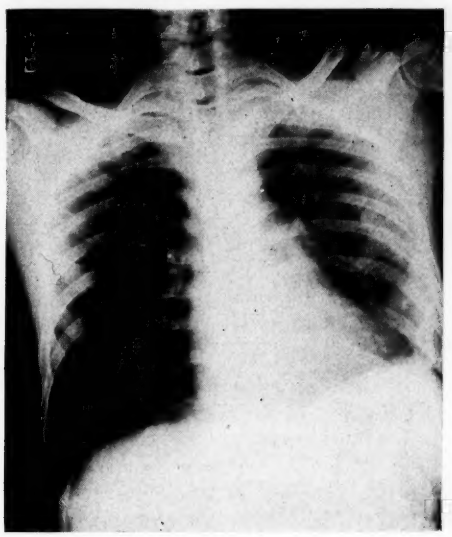

Fig. 2.

Fig. 1.—Seventh day of prontosil administration (Jan. 31, 1939).
Fig. 2.—Fourteenth day of prontosil administration (Feb. 7, 1939).

Physical signs continued to be absent until February 7, when a few fine crepitant râles were noted in the left axilla without other physical signs. X-ray at this time showed multiple areas of abnormal density throughout the left lower lobe and suggested a patchy bronchopneumonia (Fig. 2). Two weeks later x-ray films showed the left lung almost entirely involved and the appearance of an area of increased density on the right (Fig. 3). Crepitant and medium râles were heard over the areas involved without appreciable alteration of resonance, fremitus, or breath sounds. A pleuropericardial friction rub appeared, accompanied by severe precordial pain; later there was pleural friction. These signs lasted only a few days and there was no effusion. Temperature of an irregular type at moderate to high levels prevailed throughout forty days (Chart 1). Rigors occurred during the height of the process with subsequent peaks of temperature, reaching 105° F., followed by profuse sweating. Cyanosis was not noted and the degree of prostration was so slight as to be indeed remarkable. Dyspnea, paroxysmal in type, was a complaint during a few days at the height of the disease, without obvious change in the physical signs and without cyanosis, but it was relieved in the oxygen tent. Prontosil was discontinued on March 7 after forty days of ad-

ministration, and improvement rapidly ensued. A vaccine which had been given had been discontinued three weeks earlier. The physical signs in the chest cleared and x-ray films on March 25 (fifty-nine days after the onset of the pulmonary symptoms) revealed only a moderate increase in the bronchovascular markings.

On the fifty-eighth hospital day the patient complained of pain in the left groin unaccompanied by obvious physical signs. Femoral phlebitis of low grade was suspected, and this diagnosis became obvious with the development of moderate swelling of the left leg only after the patient was allowed to get up about three weeks later. Aside from this condition, the patient was apparently well at time of discharge, April 4, seventy-three days after admission.

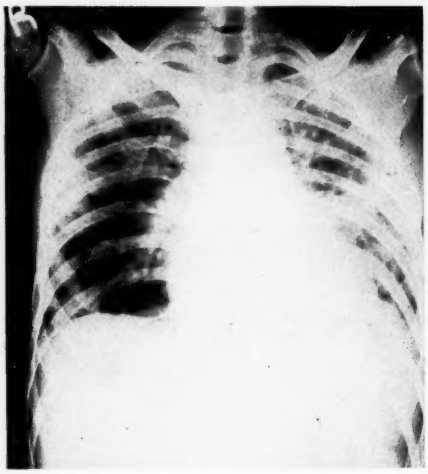

Fig. 3.

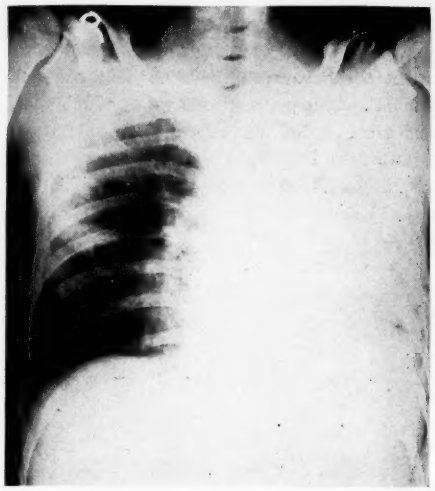

Fig. 4.

Fig. 3.—Twenty-first day of prontosil administration (Feb. 14, 1939).

Fig. 4.—Forty-second day of prontosil administration and date of discontinuance (March 7, 1939).

There was no further contact with the patient until the following January, when he reentered school at the University of Minnesota. At this time the following additional history was obtained: During July the patient took neoprontosil for a few days without apparent reason other than "general principles" (to use his own words) and very shortly developed fever and symptoms of bronchitis (?). A physician was called; he noted râles without other findings. With discontinuance of prontosil the symptoms subsided promptly. No x-ray examination was done at that time.

The general physical examination on January 15, 1940, exactly one year following the hospital admission, was essentially negative. The Mantoux test was negative to 1 mg. of old tuberculin. The x-ray of the chest was also negative (Fig. 6).

Laboratory.—The urine was negative on admission and throughout. The blood on admission showed 8,800 leucocytes and hemoglobin content 95 per cent. The differential count showed polymorphonuclear leucocytes 64 per cent, lymphocytes 26 per cent, eosinophiles 5 per cent, monocytes 4 per cent, and basophiles 1 per cent. Two weeks later the leucocytes had increased to 15,800 and continued around that level to March 6. (See Chart 1 for results of a series of blood counts.) Differential counts were not done after admission until February 26, when an eosinophile count of 33 per cent was discovered. The eosinophiles increased to 46 per cent on March 7 (the date prontosil was discontinued), and then rapidly diminished to 7 per cent ten days later. Blood cultures were repeatedly negative. Agglutination tests on admission, and at two weeks and four weeks later, were negative for *Eberthella typhi, Salmonella paratyphi;* and related organisms, *Shigella dysenteriae, Brucella melitensis,* and *Pasteurella tularensis.* Sulfanilamide determinations showed a low concentration, consistently only a trace. The leucocyte count January 15, 1940, one year following admission, was 6,100, with 1 per cent eosinophiles. *Stool examinations* were repeatedly negative for *Entamoeba histolytica* and other animal parasites or ova.

Sputum was never present. Throat culture revealed a few *B. hemolytic streptococci* on one occasion.

The Mantoux test was negative on admission and shortly before discharge.

Culture and guinea pig inoculations with stomach washings were negative for *Mycobacterium tuberculosis.*

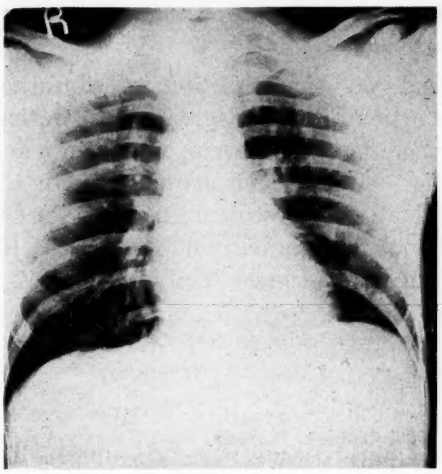

Fig. 5.

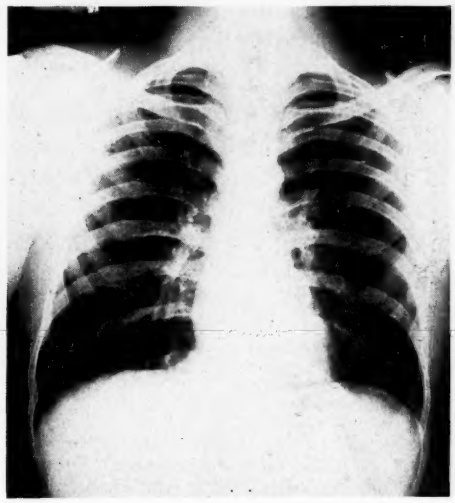

Fig. 6.

Fig. 5.—Eighteen days following discontinuance of prontosil (March 25, 1939).
Fig. 6.—One year following hospital admission (Jan. 15, 1940).

DISCUSSION

The occurrence of an unusual type of pneumonia in which massive x-ray findings stood in decided contrast with the slight degree of prostration of the patient, as well as the absence of expected physical findings, had challenged our attention even before high-grade eosinophilia was demonstrated. Eosinophilia has long been recognized as a concomitant of the allergic response. We do not fail to recognize the fact that high-grade eosinophilia is also present in other conditions, occasionally in infections. We feel that such conditions as commonly are associated with eosinophilia were satisfactorily ruled out. Moreover, we do not believe that the pneumonia observed in our patient resulted from microbic invasion. Numerous clinicians of extensive experience were puzzled by it, and bacteriologic methods failed to demonstrate an etiologic agent. Granted that usual bacteriologic methods would have failed in the discovery of a virus as the etiologic agent, clinically it was not virus pneumonia, it did not occur in the course of an epidemic, nor was there any evidence of communicability.

The fever in this case is consistent with a diagnosis of allergic pneumonia, for fever is probably the most frequent symptom of drug allergy, and furthermore, has been frequently recorded in connection with allergy to sulfanilamide. The importance of the recognition of drug allergy as a cause for fever during the treatment of a disease which is in itself accompanied by fever is obvious. The sulfanilamide exhibited in this case was a treatment for ulcerative colitis

and not under the direction of the essayists. Consent to its discontinuance resulted in coincident prompt and complete recovery. The recurrence of similar symptomatology several months later, coincident with a further trial of prontosil, was not, unfortunately, under our own observation. Nevertheless, the history strongly suggests a repetition of the same pulmonary changes we observed. The shorter duration is in keeping with a shorter period of administration of the drug.

SUMMARY

A case of massive, migrating, atypical pneumonia of unusual duration, accompanied by high fever grossly disproportional to the degree of prostration, and a marked eosinophilia, is presented. The condition developed within a few days after initial prontosil administration, continued concomitantly with the use of this drug for six weeks, and cleared up rapidly coincident with its discontinuance. A recurrence of similar pulmonary symptoms not observed by the essayists is reported by the patient following a subsequent trial of prontosil.

We are indebted to Dr. Wesley J. Spink for having called attention to many of the foreign references cited, and who also first suggested the possibility of allergic etiology.

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EFFECT OF HISTAMINASE ON THE SHWARTZMAN PHENOMENON*

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IT HAS been postulated that histamine plays an important role in the production of the Shwartzman phenomenon.[1, 2] This suggests that histaminase, the specific enzyme which inactivates histamine, might be effective in preventing or modifying the reaction.

The following experiments were carried out in an attempt to determine the effect of histaminase on the Shwartzman phenomenon.

An active extract of *B. proteus* organisms for producing the Shwartzman phenomenon was prepared by a method very similar to that described by Antopol,[3] which was based on procedures of Furth and Landsteiner[4] and Levine and Frisch.[5]

In a preliminary experiment 8 rabbits were each given a preparatory dose of 2.5 mg. of the *B. proteus* extract and a provocative or reacting dose of 0.5

*From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh, Ralph R. Mellon, M.D., Director.

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mg. Four of these animals were pretreated with histaminase.* This histaminase was standardized by the Winthrop Chemical Co., 1 unit being the amount which will inactivate 1 mg. of histamine hydrochloride. The dosages administered orally and subcutaneously varied from 6 to 12 units per kilogram and correspond to six to twelve times the daily oral dose advocated for clinical use by the Winthrop Chemical Co. The intravenous dosages varied from 4 to 30 units per kilogram and correspond to one-third to two and one-half times the doses used by Karady and Browne[6] on guinea pigs in histamine and anaphylactic shock. The results, as indicated in Table I, Series 1, showed no effect of the histaminase on the skin reaction.

As a result of numerous titrations, it was found that 0.1 mg. of the extract was effective as a preparatory dose, yet produced no gross lesion itself; and that 0.05 mg. as a provocative dose was minimal for eliciting the skin reaction in a high percentage of the animals tested.

Three more series, totaling 34 animals, were given these minimal doses of bacterial extract, and half of each series was pretreated with histaminase. It is seen from the results in Table I that again the histaminase had no striking effect.

TABLE I

RESULTS OF SHWARTZMAN REACTION WITH AND WITHOUT PRETREATMENT WITH HISTAMINASE

	SERIES 1		SERIES 2		SERIES 3		SERIES 4	
	NUMBER HISTAMINASE RABBITS*	NUMBER CONTROL RABBITS	NUMBER HISTAMINASE RABBITS†	NUMBER CONTROL RABBITS	NUMBER HISTAMINASE RABBITS‡	NUMBER CONTROL RABBITS	NUMBER HISTAMINASE RABBITS§	NUMBER CONTROL RABBITS
Died #	1	1			1			
++++	3	3						
+++			6	5	2	5	2	2
++			1	1	1		1	1
+							1	1
0			1	2	1			

\# Death occurring shortly after provocative injection of extract.
++++ Hemorrhagic skin reactions more than 4 cm. in diameter.
+++ Hemorrhagic reactions 2 to 4 cm. in diameter.
++ Reactions less than 2 cm. in diameter.
+ Very slight reactions or merely hyperemia.
0 No reaction.

*Enteric-coated histaminase capsules were given each rabbit as follows: 3 units orally five hours before the preparatory injection of extract; 3 units orally two hours after this injection; 3 units orally plus 3 units ground up with 5 c.c. of normal saline injected subcutaneously in the back four hours before the provocative injection; and 3 units subcutaneously immediately after this injection of extract. The suspensions of histaminase for injection were freshly prepared a few minutes prior to use.

†Each rabbit received tablets of histaminase as follows: 10 units ground up with 2 c.c. of normal saline injected subcutaneously six hours before the preparatory injection of extract; a similar injection one hour after the preparatory injection; and the same dose repeated one hour before the provocative injection. The suspensions of histaminase were freshly prepared a few minutes prior to use.

‡Each rabbit received 10 units of histaminase (injectable) powder dissolved in 10 c.c. of saline and injected intravenously fifteen to thirty minutes before the provocative injection of extract.

§Each rabbit received the supernatant fluid from 75 units of histaminase tablets ground finely in a mortar with 10 c.c. of normal saline, centrifuged, and injected intravenously fifteen to thirty minutes before the provocative injection of extract.

Since it requires twenty-four hours for 1 unit of histaminase to inactivate 1 mg. of histamine in vitro, it may be anticipated that the Shwartzman reaction develops too rapidly for histaminase to inactivate any histamine that may be released. The report of Karady and Browne[6] appears to indicate that histamin-

*A small portion of the histaminase used in these experiments was furnished by the Winthrop Chemical Co., Rensselaer, N. Y., and the remainder, prepared by the same concern, was purchased in open market.

ase acts rapidly in vivo. However, using the same technique and a larger group of animals, Knoll[7] was unable to confirm their work on anaphylactic shock.

An alternative worthy of consideration is that histamine-like substances rather than histamine itself may be responsible in the Shwartzman reaction. In this case, of course, it would not be expected that histaminase would have any effect on the reaction.

It is also possible that the increase in histamine content of the skin in the Shwartzman phenomenon is secondary, and an effect rather than a cause.

SUMMARY AND CONCLUSIONS

Little or no effect on the Shwartzman phenomenon was noted by pretreatment with histaminase administered orally, subcutaneously, or intravenously in large doses. Either histamine plays little part in the phenomenon or histaminase does not inactivate sufficiently the histamine released.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH HEMOLYTIC ANEMIA (MARCHIAFAVA-MICHELI SYNDROME)*

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THE syndrome of paroxysmal nocturnal hemoglobinuria was recorded first by Marchiafava and Nazari in 1911.[1, 2] In 1931 Micheli[3] reviewed all the cases published and added one to the literature. Since then other cases have been recorded by Hamburger and Bernstein,[4] Witts,[5] Scott, Robb-Smith, and Scowen,[6] Dacie, Israels, and Wilkinson,[7] and Ham.[8] These authors have described the clinical, pathologic, and laboratory features which have established nocturnal hemoglobinuria as a specific disease.

From a clinical consideration of this disease, certain essential features are recognized. In the cases reported, it occurred most frequently during the second and third decades of life and, following a protracted course, terminated in death.

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Hemoglobinuria was the predominant sign and occurred almost always at night, frequently accompanied by vague abdominal pains. The course of the illness was characterized by recurring attacks of fever which were usually intermittent, a predisposition to venous thrombosis and phlebitis, and a slight icteric tint to the sclerae and skin. By the use of suitable reagents, hemosiderin and hemoglobin could be demonstrated in the urine. In a few cases slight enlargement of the spleen was reported.

The blood changes in nocturnal hemoglobinuria are most striking. In the cases reported in the literature, between one and three million red blood cells per cubic millimeter were noted. In most instances the cells were slightly larger than normal, so that the anemia was usually macrocytic; in others, it was normocytic. The hemoglobin was reduced to between 18 and 50 per cent of normal; hyperchromia was noted in some cases and hypochromia in others. Whenever hemolysis had occurred recently, hypochromia and consequently a lower color index were observed. In general, therefore, there was a tendency to slight macrocytosis (M.C.V. 100 cubic microns) and hypochromia of the red blood cells (M.C.H.C. 27 per cent). In all instances the anemia was refractory to treatment. The resistance to hypotonic salt solution was within normal range, and an examination of so-called "wet preparations" failed to show the presence of spherocytes. The outstanding feature was a persistent reticulocytosis. Invariably there was a leucopenia with a relative lymphocytosis and a moderate thrombocytopenia. Examination of the sternal bone marrow by either puncture or biopsy showed hyperplasia of the erythropoietic elements.

The etiology of hemoglobinuria is somewhat obscure. From a study of the reports in the literature it was apparent that, in contrast to familial hemolytic icterus, a familial predisposition did not exist in patients with nocturnal hemoglobinuria. Dacie, Israels, and Wilkinson,[7] and Ham,[8] were able to show by experiments both in vivo and in vitro that increased carbon dioxide tension was of critical importance for the production of hemolysis.

The following case of nocturnal hemoglobinuria is presented, first, because of the rarity of the condition and, secondly, to record the results of in vitro studies showing increased hemolysis of red blood cells in media at an acid pH. The patient had an illness of several years' duration characterized by anemia, vague abdominal pain, thrombophlebitis, and hemoglobinuria occurring at night.

REPORT OF A CASE

History.—A. J., an American woman, single, aged 43 years, was first admitted to the University of California Hospital on May 31, 1938. She complained of weakness and stated that she was known to have been pale and anemic for at least six years. Five years previous to her present entry she had suffered profuse uterine bleeding which was relieved by salpingo-oophorectomy and hysterectomy. Despite the cessation of menses, however, she continued to be anemic, and on two occasions obtained temporary relief from transfusions. In 1935 she had an attack of phlebitis in the right leg which persisted for several months. She had a similar attack in August, 1937, and in November, 1937, the veins of the left leg became involved. At this time she first noticed that her skin had a slightly yellowish tinge and experienced vague abdominal pains which were most marked at night. She then noticed that the urine passed on arising in the morning was very dark. In December, 1937, her physician told her she had had a hemorrhage from her kidneys. This episode lasted about one week and recurred about a month later.

Physical Examination.—On entry to the hospital the patient was well developed, moderately well nourished, looked tired and pallid, and had slightly icteric sclerae. The ears,

nose, throat, and lymph nodes presented no remarkable abnormalities. The teeth were carious, but the gums were firm and showed no bleeding points. There were no signs of disease in the heart or pulmonary parenchyma. The blood pressure was 122 systolic and 68 diastolic. Neither the spleen nor the liver could be felt on palpation. There was slight pitting edema of the ankles.

Laboratory Data. Examination of the blood showed red blood cells 1,970,000 per cubic millimeter, hemoglobin 56 per cent (7.8 Gm. Sahli), and leucocytes 2,200 per cubic millimeter. The differential count showed polymorphonuclear leucocytes 61 per cent (29 per cent filamented, 32 per cent nonfilamented forms), eosinophiles 3 per cent, basophiles 2 per cent, and small lymphocytes 34 per cent. The reticulocytes were 3 per cent. The volume of packed cells was 25 c.c. per 100 c.c. of blood. The mean corpuscular volume was 126 cubic microns, the mean corpuscular hemoglobin was 39 micromicrograms, and the mean corpuscular hemoglobin concentration was 31 per cent. The color index was 1.39, the volume index 1.47, and the saturation index 0.93. The fragility of the red blood cells established with the usual hypotonic saline solutions was within normal range. The rate of sedimentation of the erythrocytes was 24 mm. in one hour (corrected Wintrobe method). The reactions to the Wassermann and Kahn tests of the blood were negative. The phenolsulphonphthalein excretion tests showed normal renal function. No abnormalities of the kidneys were observed in the retrograde pyelograms. The determination of liver function by using the rose bengal dye showed normal excretion. The total blood serum protein was 5.82 Gm. per 100 c.c., of which 3.54 Gm. was albumin and 2.28 Gm. was globulin; the ratio of albumin to globulin was 1.55. Absence of hydrochloric acid from the gastric secretion was demonstrated by parenteral injection of histamine. Roentgenograms of the gastrointestinal tract and of the long bones revealed no abnormalities.

Bone marrow removed from the sternum for biopsy was examined by the usual technique of Zenker's fixation and staining with hematoxylin and eosin. It showed a general hyperplasia of both erythropoiesis and myelopoiesis. Large numbers of cells containing pyknotic nuclei and hemoglobin, and an abundance of younger forms with basophilic cytoplasm and large, deeply stained nuclei were observed. These were considered to be normoblasts and erythroblasts. No megaloblasts were seen. Only a few myeloblasts were noted, but there were large numbers of cells ranging from the stage of myelocytes to the fully developed polymorphonuclear leucocytes. The diagnosis was moderate generalized hyperplasia of erythropoiesis and leucopoiesis.

Course of Illness.—Repeated examination of the blood showed only little change in the red blood cells and their hemoglobin content, but the reticulocytes varied in number from 3 to 10 per cent of the total count. Ten days after entry the patient was discharged from the hospital to her physician who was advised to give her liver extract potent in pernicious anemia by injection and large doses of iron by mouth. In spite of this treatment the anemia persisted.

Second Entry. On August 19, 1938, the patient was readmitted to the hospital complaining that she had secreted dark urine for ten days. She stated that, except for weakness, she had felt about as usual until the present episode, when she began having chilly sensations, vague abdominal pains, and a daily afternoon rise in temperature to 100° F. She passed large quantities of bright red urine, which upon analysis by her physician was reported to contain blood.

TABLE I

	1:30 A.M.	7 A.M.	9 A.M.	NOON	3 P.M.	9 A.M.
Hemoglobin	++	++	0	0	0	0
Bile	0	0	0	0	0	0
Urobilin	1:10	1:50	1:10	0	0	0

The physical examination was essentially the same as previously reported.

Laboratory Data. The urine showed no bile, albumin, sugar, or casts. Urobilinogen was noted in a concentration of 1:50 dilution units in specimens obtained at 7 A.M., and 1:10 in specimens obtained at noon. Hemoglobin was present in specimens of urine obtained at

1:30 A.M. and 7 A.M., but was absent in specimens obtained after 9 A.M. (see Table I). Because of the presence of the hemoglobin in the urine, the patient's illness was recognized as paroxysmal nocturnal hemoglobinuria of the Marchiafava-Micheli type.

Special Procedures. A set of serologic experiments was arranged similar to those performed by Dacie, Israels, and Wilkinson.[7] Serum oxalated plasma and heparinized plasma were used as hemolytic media. Duplicate series were set up with serum and plasma of a normal person of the same blood grouping (Moss II).

1. The patient's red blood cells, after having been carefully washed with physiologic salt solution, were added to tubes containing the patient's serum and plasma, and to tubes containing serum and plasma obtained from a normal person. The normal person's red blood cells were added to a series of tubes containing similar media. All tubes were placed in a water bath at a temperature of 37° C. One-tenth cubic centimeter of packed red blood cells was added to 1.0 c.c. of plasma or serum in each instance. The results are summarized in Table II. It is to be noted that a slight amount of hemolysis occurred when the patient's cells were mixed with her own and with normal serum. There was no hemolysis of the normal cells in similar preparations.

TABLE II

SHOWING A MARKED DEGREE OF HEMOLYSIS OF THE PATIENT'S RED BLOOD CELLS WHEN THE MEDIA ARE SLIGHTLY ACIDIFIED

	PHYSIOLOGIC SALT SOLUTION	PATIENT'S SERUM	PATIENT'S OXALATED PLASMA	PATIENT'S HEPARINIZED PLASMA	NORMAL SERUM	NORMAL OXALATED PLASMA	NORMAL HEPARINIZED PLASMA
Patient's cells	0	Slight	0	0	Slight	0	0
Normal cells	0	0	0	0	0	0	0
Patient's cells { CO_2 in solution	0	++++	+	+++	+++	+	++
Normal cells { CO_2 in solution	0	0	0	0	0	0	0
Patient's cells { chilled serum		0	0		0	0	
Normal cells { chilled serum		0	0		0	0	
Patient's cells { chilled serum CO_2 in solution		+++			+++	+	
Normal cells { chilled serum CO_2 in solution		0	0		0	0	

2. Duplicates of the above procedures were set up, and carbon dioxide in 5 per cent dilution was bubbled through the serum and plasma. The results are also summarized in Table II. It is to be noted that in the acidified media definite hemolysis occurred in all solutions containing the patient's cells. In the tubes containing plasma (of the normal person and the patient), which had been treated with sodium oxalate to prevent coagulation, hemolysis was less marked. The hemolysis which occurred in the tubes containing normal cells with the serum of the normal person and the patient was negligible.

3. To determine whether or not there was a positive Donath-Landsteiner reaction, the serum and oxalated plasma were chilled prior to the addition of the red blood cells and the carbon dioxide gas. It is apparent from the data recorded in Table II that hemolysis was not increased by the low temperature.

4. In another series of experiments serum was heated to 56° C. The cells of the patient and those of the normal person were added to this serum. No hemolysis occurred even after bubbling the carbon dioxide gas through the serum. The hemolytic activity of the serum was not restored by the addition of complement. These results are summarized in Table III. This demonstrated that evidently the lysin was thermolabile and that the previous reactions were not due to a complement-fixation mechanism.

TABLE III

HEMOLYTIC ACTIVITY OF THE SERUM IS DESTROYED WHEN HEATED TO 56° C.

	PATIENT'S SERUM + CO_2	PATIENT'S SERUM HEATED 56° C.	PATIENT'S SERUM HEATED + CO_2	PATIENT'S SERUM HEATED + COMPLEMENT	PATIENT'S SERUM HEATED + COMPLEMENT + CO_2	NORMAL SERUM HEATED	NORMAL SERUM HEATED + CO_2	NORMAL SERUM HEATED + COMPLEMENT	NORMAL SERUM HEATED + COMPLEMENT + CO_2
Patient's cells	++++	0	0	0	0	0	0	0	0
Normal cells	0	0	0	0	0	0	0	0	0

Treatment.—Since in vitro experiments showed an increased hemolysis at an acid pH, it was thought that the administration of large amounts of alkali might favorably alter the lytic mechanism. Accordingly, the patient was given a sufficient amount of sodium bicarbonate (4.0 Gm. four to six times a day) to render the urine alkaline (pH 7.6). However, during this treatment the patient had more severe abdominal pain and hemoglobinuria than before.

The oral administration of large doses of iron, the parenteral administration of liver extract effective in the treatment of pernicious anemia, together with a high protein diet, autolyzed yeast, and a period of multiple transfusions failed to increase the production of hemoglobin or red blood cells.

DISCUSSION

The diagnosis of our patient presented a considerable problem. She had an anemia probably of hemolytic origin accompanied by slight icterus and reticulocytosis. The degree of the anemia, the macrocytosis, the leucopenia, and the slight thrombocytopenia were suggestive of pernicious anemia. However, the failure of the bone marrow to respond to liver therapy removed this possibility from further consideration. The resistance of the red blood cells to hypotonic salt solution within normal range, the negative family history, and the absence of an enlarged spleen excluded the possibility of hemolytic icterus of familial origin. Studies of the urine during the patient's first hospitalization were inadequate, but during the second hospitalization discovery of the nocturnal emission of hemoglobin led to the recognition of the anemia as the hemolytic type described by Marchiafava.[1]

No adequate explanation can be offered for the abnormal hemolytic mechanism in this patient. Special procedures were carried out according to the methods described by Dacie, Israels, and Wilkinson,[7] and Ham.[8] Our results

were comparable to theirs. After adding the patient's washed red blood cells to her serum and to normal serum, a slight degree of hemolysis was observed. When the media were acidified with carbon dioxide, the hemolysis was greatly increased. When the serum was heated to 56° C., the hemolytic activity was destroyed. It seems apparent from these studies that the patient's red blood cells were sensitized to some lysin present in her own serum and in normal serum. The role that hydrogen-ion concentration of serum or plasma may play in the production of the lysin deserves further investigation.

SUMMARY

The clinical and laboratory findings in a patient with nocturnal hemoglobinuria are presented. The possible significance of increased hydrogen-ion concentration in the production of lysin is briefly discussed.

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Since this paper was written, the patient died (Feb. 19, 1940). The cause of death was reported to be due to cerebral thrombosis. The spleen was normal in size.

FALSE POSITIVE SEROLOGIC REACTIONS FOR SYPHILIS IN INFECTIOUS MONONUCLEOSIS*

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MANY diseases besides syphilis may be accompanied by positive serologic reactions ordinarily considered diagnostic of syphilis. In malaria, during the acute stage, there is very frequently a transiently positive Wassermann or flocculation reaction.[1] Trypanosomiasis, relapsing fever, and ratbite fever are sometimes attended by temporarily positive tests.[2] Some patients with lymphogranuloma venereum[3] and with kala-azar[4, 5] also show such reactions. Approximately 60 per cent of leprous patients have positive serology; and in yaws positive complement fixation and flocculation reactions occur as regularly as in syphilis.[6] Christie[7] has recently emphasized that many infants born to Wassermann-positive syphilitic mothers have positive serology at birth. In most instances this disappears without treatment and, therefore,

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represents merely the passage of reagin through the placenta. Serologic tests, in various degrees of positivity, have also been reported following vaccination for variola,[8, 9] and in cases of malignant neoplasms, jaundice, hyperpyrexia, pulmonary tuberculosis, typhoid, typhus, scarlet fever, "herpes genitalia," gonorrhea, "angina," bronchitis, and most often in upper respiratory infections and lobar pneumonia.[10-13] However, the following statement is made by no less an authority than Eagle[6]: "The statistical reports of the past 10 to 15 years make it reasonably certain that neither tuberculosis, diabetes, pregnancy, anesthesia, malignancy, jaundice, fever, nor hypercholesterolemia predisposes to false-positive reactions."

A small number of references to the occurrence of false positive serologic reactions in infectious mononucleosis have appeared in the literature of the past decade. Weber[14] in 1930, and Weber and Bode[15] in 1931, reported 3 such cases with positive Wassermann and Meinicke tests. Gooding[16] in 1931 stated that 16 of 27 patients with infectious mononucleosis gave either a positive or a doubtful Wassermann reaction, and that those patients who showed a negative Wassermann test in most instances showed a positive Kahn test. He stated further that the Wassermann reaction returned to negative spontaneously six to eight weeks after the acute stage. Unfortunately, details concerning the positive tests and the return to negative are most meager, so that it is not possible to say exactly how many of these cases should actually be included. One positive serologic test followed by repeated negative tests should not entitle a case to be included in our discussion, since this may represent merely a technical or clerical error.

Hatz[17] in 1938 published one clear-cut case with strongly positive transient Wassermann reactions and simultaneously negative Kline tests. The next year Saphir[18] reported one case with strongly positive Wassermann and Kahn reactions on several occasions, which became negative within several weeks without antisyphilitic treatment. Also in 1939 Sadusk[19] described a patient whose blood gave temporarily positive reactions with the Kahn and the cholesterolized Wassermann antigens, and negative reactions with the alcoholic antigen. This case was definite; but the author incidentally mentions 2 others which are doubtful, since in both instances "weakly positive" reactions, reported on only one occasion, were not confirmed.

Bernstein[2] published an excellent account in 1938 of 6 cases in which the Wassermann or the Eagle flocculation test, or both, became temporarily positive during the course of infectious mononucleosis. Two of these cases had previously been reported by him.[20] Of the 6 cases, one had a "doubtful" Eagle, a negative Wassermann, and no further tests. This case should, in my opinion, be excluded from such a series. In a recent monograph on infectious mononucleosis, Bernstein[21] states that 8 cases with false positive serology have been observed by him at the Johns Hopkins Hospital, thus adding 2 new cases to those previously reported.[2] The cases described by Löhe and Rosenfeld,[22] Radford and Rolleston,[23] Wawersig,[24] and Asahina[25] should all be left out of any tabulation of false positive serologic reactions in infectious mononucleosis for one of two reasons: either the diagnosis of infectious mononucleosis was not certain or syphilis was not definitely excluded.

The incidence of false positive serologic reactions in infectious mononucleosis is difficult to determine with any degree of accuracy. According to the combined series of Gooding,[16] Saphir,[18] Sadusk,[19] Bernstein,[21] and myself, there were 29 patients with false positive reactions out of 183 patients with infectious mononucleosis who had serologic tests for syphilis performed. This gives an incidence of 15.8 per cent. But if Gooding's series—which is so contrary to the experience of all other clinicians—were excluded, the incidence would be only 8.4 per cent.

In contrast to these figures are the unpublished observations of several careful investigators[26] who, although they have heard indirectly of 2 or 3 unpublished cases of false positive reactions in infectious mononucleosis, have not personally observed a single such case in a combined experience of between 150 and 250 serologically tested patients. The actual incidence of recognized false positive reactions would, therefore, seem to be between 2 and 10 per cent. But as Bernstein[2] has stated: "It should be emphasized that the evanescent character of these positive serological reactions mitigates against the likelihood of recognizing all the instances in which they occur. It is reasonable to assume, therefore, that if these serological tests were carried out more regularly and persistently, the incidence of positive reactions encountered would be considerably higher."

Among 82 proved cases of infectious mononucleosis that I have investigated during the past three years, there were 3 with false positive serology. Sixty-four of the 82 had at least one serologic test for syphilis performed during the acute stage of the illness, and 32 of these had such tests on 2 or more occasions. The diagnosis of infectious mononucleosis was established by the clinical course, typical blood smears, and sheep cell agglutination tests. Two of the patients were known to have congenital syphilis with negative serology following antisyphilitic treatment. It is interesting to note that in neither case did the Wassermann reaction revert to positive during the course of infectious mononucleosis.

TABLE I

SEROLOGIC REACTIONS IN CASE 1

DAY OF ILLNESS	WASSERMANN (ALCOHOLIC)	WASSERMANN (CHOLESTEROL)	KLINE	SHEEP CELL AGGLUTINATION TITER
11	Negative	2+	Negative	1:32
22	1+	4+	Negative	----
29	Negative	2+	Negative	----
32	Negative	1+	Negative	----
42	Negative	Negative	Negative	1:448
150	----	----	----	1:112
360	----	Negative	----	1:14

CASE REPORTS

CASE 1.—The first patient with false positive serology was a male pharmacy student, aged 22 years, who had had high fever, several chills, severe frontal headache, profuse sweats, marked fatigue, and general malaise for ten days prior to admission. He was sent to the hospital with the tentative diagnosis of typhoid fever. On admission he was found to have a markedly congested pharynx, slight enlargement of the spleen, marked hyperesthesia of the skin over the abdomen, and tenderness in the left lower quadrant. There was considerable

enlargement of the axillary, inguinal, and epitrochlear lymph nodes, all of which were firm and very tender. There were also a few small nontender left posterior cervical and bilateral anterior cervical nodes. The blood smear showed 12 per cent polymorphonuclear leucocytes and 88 per cent lymphocytes. The total white blood cell count was 13,400; and the red blood cell count and hemoglobin were within normal limits. On the fourteenth day of illness the monocytes numbered 11 per cent, and in the fifth week 40 per cent. Agglutination tests for typhoid and paratyphoid A and B infections were negative on two occasions. The results of the other serologic tests are shown in Table I.

CASE 2.—A male interne, aged 25 years, was admitted after having had a sore throat, cough, chills, and fever for one day. Examination revealed only a congested nasal mucosa and pharynx. The initial diagnosis was grippe. By the sixth day of illness he had a severe cough, high fever, mild conjunctivitis, and slight enlargement of the posterior cervical lymph nodes bilaterally. A positive sheep cell agglutination test and characteristic blood smears soon established the diagnosis of infectious mononucleosis. On the fourth day of illness the white blood cells were 11,400; polymorphonuclear leucocytes were 78 per cent, of which 27 were immature; lymphocytes 10 per cent; monocytes 12 per cent. On the ninth day the white blood cells numbered 15,000; polymorphonuclear leucocytes were 73 per cent, of which 40 were immature; lymphocytes 7 per cent; monocytes 4 per cent; eosinophiles 3 per cent; abnormal cells of the mononuclear series 13 per cent. Eight weeks after the onset a smear showed the following: polymorphonuclear leucocytes 39 per cent; lymphocytes 8 per cent; monocytes 9 per cent; eosinophiles 2 per cent; abnormal cells of the mononuclear series 42 per cent. The second blood smear was suggestive of infectious mononucleosis; subsequent ones were typical. The results of the various serologic tests performed in four different laboratories (a, b, c, d) are shown in Table II.

TABLE II

SEROLOGIC REACTIONS IN CASE 2

DAY OF ILLNESS	WASSERMANN (ALCOHOLIC)	WASSERMANN (CHOLESTEROL)	KLINE	MEINICKE	EAGLE AND NEW EAGLE	SHEEP CELL AGGLUTINATION TITER
9	--	4 + (a)	--	Negative (b)	--	1:56 (b)
19	--	2 + (a)	--	Negative (b)	--	1:112 (b)
32	4 + (b)	4 + (a)	Negative (a)	Negative (b)	--	1:28 (b)
41	4 + (b) 4 + (c)	4 + (a) 3 + (c) 18 U. Positive (d)	Negative (a) Negative (c) Doubtful (d)	Negative (b)	Negative (d)	1:28 (b)
54	± (b) 4 + (c)	4 + (a) Negative (b) ± (c) 6 U. Positive (d)	Negative (a) Negative (c)	Negative (b)	Negative (d)	--
72	2-3 + (b) 1 + (c)	Negative (a) Negative (c)	4 + (c)	Negative (b)	--	1:112 (b)
89	± (b) Negative (c)	Negative (a) ± (b) Negative (c)	Negative (c)	Negative (b)	--	1:56 (b)
103	Negative (c)	Negative (a) Negative (b) Negative (c) Negative (d)	Negative (a) Negative (c) Negative (d)	Negative (b)	Negative (d)	--

CASE 3.—A male child, aged 2½ years, was seen in the pediatric clinic after having had fever and anorexia for one week. A pin-point vesicular eruption was present over the entire body; the tonsils were enlarged and congested; there was a generalized lymphadenopathy; and the temperature was 104.2° F. Varicella was diagnosed and the child was

sent home. Two weeks later he was admitted to the hospital, since the fever, marked fatigue, and anorexia had continued, and there were in addition occasional vomiting and enuresis. Examination revealed temperature 100.6° F., pulse rate 104, respirations 24. There was a left catarrhal otitis media, injected tonsils and pharynx, and generalized lymphadenopathy.

A Widal test, a tuberculin test, and several urine examinations were all negative. A blood count, done the day before admission, showed white blood cells 17,000, polymorphonuclear leucocytes 4 per cent and lymphocytes 96 per cent. The following day the figures were hemoglobin 88 per cent, red blood cells 4,400,000, white blood cells 4,200, polymorphonuclear leucocytes 50 per cent, and lymphocytes 50 per cent. Four days later the smear showed polymorphonuclear cells 10 per cent, lymphocytes 29 per cent, monocytes 16 per cent, eosinophiles 1 per cent, and abnormal cells of the mononuclear series 44 per cent. The serologic reactions reported by several laboratories are summarized in Table III, wherein the headings "Kline" and "Eagle" refer to the Kline diagnostic and the Eagle microflocculation tests.

TABLE III

SEROLOGIC REACTIONS IN CASE 3

DAY OF ILLNESS	WASSERMANN (ALCOHOLIC)	WASSERMANN (CHOLESTEROL)	KLINE	MEINICKE	EAGLE	KAHN	SHEEP CELL AGGLUTINATION TITER
26	Negative (b) Negative (c)	4 + (a) Negative (c)	4 + (c) 3 + (e)	Negative (b)	--	--	1:28 (b) 1:32 (c) 1:14 (e)
29	Negative (c)	Negative (c)	2 + (c)	--	--	--	--
33	Negative (b) Negative (c)	3 + (a) Negative (c) Doubtful (d) Negative (e)	4 + (a) Negative (c) Doubtful (d) Negative (e)	Negative (b)	Doubtful (d)	Negative (e)	1:7 (e)
39	Negative (b) Negative (c)	Negative (c) Doubtful (d)	2 + (c) Doubtful (d) Negative (e)	Negative (b)	Positive (d)	Negative (e)	1:14 (e)
49	Negative (c)	1 + (a) Negative (c) Negative (d)	Negative (a) Negative (c) 1 + (e)	Negative (b)	Doubtful (d)	2 + (e)	1:7 (e) 1:112 (b)
63	Negative (c)	Negative (a) Negative (c)	Negative (c)	Negative (b)	--	--	1:28 (b)
97	Negative (c)	Negative (c)	1 + (c)	--	--	--	1:56 (b)

DISCUSSION

What is the significance of a positive Wassermann or flocculation reaction during the course of infectious mononucleosis? In the first place, the patient may have syphilis, in which case the tests will remain positive. Secondly, the positive report may represent a technical error, in which case all subsequent tests will be negative.[27] Thirdly, the result may be and usually is a biological false positive reaction. It may happen that a positive Wassermann report is obtained before the diagnosis of infectious mononucleosis has been definitely made or even considered. One should, therefore, observe the dictum of Bernstein: "Whenever, in the course of any disease a Wassermann, suspected of being falsely positive, is encountered, a Paul-Bunnell [sheep cell agglutination] test should be performed." Even if the latter is negative, the patient may still have infectious mononucleosis.[28]

Krag and Lonberg,[12] on the basis of a very large serologic experience, state: "If the positive [Wassermann] reaction at the first retest is constant and a thorough clinical-anamnestic investigation is completely negative, nothing

definite can be decided at once; an observation period of 3 to 6 weeks is necessary, as an unspecific reaction must have a chance to reveal itself." Moore[27] in his excellent monograph on syphilis gives some examples of conflicting results on different samples of sera from the same patient, and concludes that several successive positive reports from different laboratories warrant the diagnosis of syphilis. Moore, and even Krag and Lonberg, do not give the patient quite enough benefit of the doubt, as Case 2 in this paper exemplifies. Various serologic tests for syphilis were still positive in this patient as long as ten weeks after the onset of the disease. In most of the reported cases the false positive tests returned to normal within nine weeks. Two of Christie's nonsyphilitic patients with transplacental passage of reagin from syphilitic mothers had false positive reactions for eighty-six days before the first negative test was obtained.[7] If one feels reasonably certain that the tests are falsely positive, I believe that the patient deserves the chance to have his blood tested for at least twelve weeks before he is subjected to any antisyphilitic treatment. A point to be stressed is that more than one negative serologic report should be obtained without specific treatment in order to rule out syphilis and class the reaction as falsely positive. Several recent articles and editorials[29-32] have summarized the present status of biologic false positive serologic tests for syphilis, and the methods of recognizing them.

TABLE IV

FALSE POSITIVE SEROLOGY IN INFECTIOUS MONONUCLEOSIS

AUTHOR	NO. CASES	RESULTS OF SIMULTANEOUS TESTS
Weber and Bode[15]	3	Positive Wassermann—Negative Meinicke Positive Wassermann—Positive Meinicke Negative Wassermann—Positive Meinicke
Gooding[16]	16 (?)	Positive Wassermann—Negative Kahn Negative Wassermann—Positive Kahn Positive Wassermann—Positive Kahn (?)
Hatz[17]	1	Positive Wassermann—Negative Kline
Saphir[18]	1	Positive Wassermann—Positive Kahn
Sadusk[19]	1	Positive Wassermann—Positive Kahn Negative Wassermann—Positive Kahn
Bernstein[21]	8	Positive Wassermann—Negative Eagle Positive Wassermann—Doubtful Eagle Doubtful Wassermann—Negative Eagle Positive Wassermann—Positive Eagle Negative Wassermann—Doubtful Eagle
Kaufman	3	Positive Wassermann—Positive, doubtful, negative Kline Doubtful Wassermann—Positive, negative Kline Negative Wassermann—Positive, doubtful Kline Positive, doubtful Wassermann—Negative Meinicke, Eagle Positive Eagle—Doubtful, negative Wassermann Positive Eagle—Doubtful, negative Kline Positive Kline—Negative Meinicke, Kahn Doubtful Kahn—Negative Wassermann, Kline Positive Wassermann—Negative Wassermann Positive Kline—Negative Kline

A study of the individual cases of infectious mononucleosis with false positive serologic tests reported in the literature, ([2, 14-20, 33] present series) fails to reveal anything characteristic about them in the clinical course, the blood counts or smears, or the sheep cell agglutination tests. There does not seem to be any

quantitative relationship between the heterophile antibodies, which give rise to the Paul-Bunnell reaction, and the substances responsible for the positive complement-fixation test (Wassermann) or for the various flocculation tests (Kline, Kahn, Eagle, Meinicke, etc.). As the cases in this paper illustrate, the same serum may give positive reactions with some tests for syphilis and negative reactions with other tests; different laboratories using presumably the same test may get totally different results with the same serum; and a positive test may be negative on the next examination, whereas a test previously negative may become positive. False positive reactions do not seem to be more frequently encountered with one serologic test than with another (Table IV), except in so far as certain tests, such as the Wassermann, are more commonly employed.

What is the cause of the false positive serologic reactions for syphilis occasionally noted during the course of infectious mononucleosis? According to Eagle's authoritative book on the laboratory diagnosis of syphilis,[6] there is likely to be some effect of native amboceptor (antibody for sheep red blood cells) on the Wassermann test. "Whereas small amounts of native amboceptor tend to give negative Wassermann reactions because hemolysis is accelerated, large amounts of native amboceptor, such as those usually observed in infectious mononucleosis, tend to cause such massive agglutination of the red blood cells as to retard hemolysis and therefore give a false positive result."[34] However, the possibility of a false positive flocculation test (Kline, Kahn, Eagle, Meinicke, etc.) being due merely to native amboceptor is clearly excluded, since red blood cells are not employed in this type of test. "The sera giving false-positive reactions apparently contain some reagin-like factor which reacts with beef heart lipoid in a manner indistinguishable from syphilitic serum."[34]

The etiologic agent in infectious mononucleosis is at present unknown. Perhaps the occurrence, even though rarely, of false positive serologic tests for syphilis may offer a clue. Bernstein[2] has suggested that "the causative agent in infectious mononucleosis may contain a substance having antigenic properties in common with the *Treponema pallida*; or else this causative agent may liberate from the tissues of the patient a reagin-like material. The fact that the majority of the diseases which may be associated with a positive Wassermann are caused by protozoa [including spirochetes as protozoa] would suggest that the causative agent of infectious mononucleosis may likewise be a protozoön."

SUMMARY

1. A review of the literature of false positive serologic tests for syphilis during the course of infectious mononucleosis has been presented.

2. Three additional cases were observed among 64 patients who had Wassermann tests performed.

3. The suggestion is made that patients should not be treated for syphilis until serologic tests, *suspected of being falsely positive*, have had a sufficient chance—about twelve weeks—to revert to negative.

4. The significance of a positive Wassermann or similar reaction during the course of infectious mononucleosis has been discussed.

I wish to express my sincere thanks to the following for their kind aid: Doctors Alan Bernstein, Israel Davidsohn, Harry Eagle, Stephen Gumport, John Koopman, Perry Manheims, Leo Spiegel, and Annis Thomson.

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EXPERIMENTAL UTEROTUBAL INSUFFLATION*

Further Investigations

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IN A PREVIOUS communication[1] there were described some elementary features of uterotubal physiology as determined by insufflation of gas according to the method of Rubin. Uterotubal kinetics are analogous to those of other smooth muscle organs in that they are affected by the autonomic nervous system.

The present experiments were undertaken to evaluate the influence exerted by the extrinsic nerves upon the Fallopian tubes and uteri of rabbits, as determined by means of transuterine insufflation of gas. The influence of induced ovulation was also studied.

The female rabbit has a double uterus ending in a single vagina. Each uterus and its tube lie at the free end of a thin, highly vascularized mesentery. The uterine and ovarian vessels form multiple arcades, and anastomose close to the free border of the mesentery. The extrinsic nerves to the pelvic genitals pass through the mesentery, lying either free or in close conjunction to the vessels. There is probably a dual, anatomical innervation with one group of nerves following the ovarian and the other the uterine vessels.[2]

METHOD

Young female rabbits, weighing 2.2 to 5.4 pounds, were insufflated by the technique previously described. The effect of nerve block as induced by infiltration of the uterine or tuboovarian mesentery with 2 per cent procaine hydrochloride was studied. Direct electrical stimulation of the mesentery was attempted, but the contractions of the smooth muscle in the mesentery itself rendered the resultant kymographic tracing difficult to interpret. Transection of the spinal cord was found to be so shocking as to invalidate conclusions at this time. An attempt was made to divide the mesentery, leaving the blood supply intact, but the operation was difficult to perform without introducing mechanical factors and at the same time it was impossible to eliminate the presence of undivided nerve fibers traveling in the vascular sheaths.

EXPERIMENTAL FINDINGS

The features of the curve that are particularly emphasized are: (1) Primary pressure peak—level at which gas enters the abdominal cavity. (2) Plateau level—general level of pressure during insufflation. (3) Small waves of fluctuation in pressure during insufflation.

Nerve Block of the Uterine Mesentery.—In 11 animals 1.0 to 1.5 c.c. of procaine was injected throughout the uterine mesentery from the cervix to the uterine cornu. Four animals were observed without any systemic anesthesia,

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and in the other 7 efficient narcosis was elicited by intravenous nembutal. After a preliminary control insufflation and when a fairly constant plateau level of insufflation was observed, the procaine was injected. Changes in the nature of the curve appeared within three to sixty seconds after injection.

TABLE I

PLATEAU PRESSURE LEVEL OF CONTROL	PLATEAU LEVEL AFTER PROCAINE	FALL
mm. Hg	mm. Hg	mm. Hg
112	58	54
90	45	45
75	50	25
72	48	24
70	58	12
57	28	29
55	45	10
50	45	5
48	25	13
45	42	3
45	40	5

1. Effect upon plateau level of insufflation. This level fell in all 11 animals. The range of fall was between 3 and 54 mm. Hg, and the amount of fall was less as the control plateau level approached 45 mm. Hg (Figs. 1 and 2).

2. Effect upon the character of the small waves of pressure.

(a) In 7 animals there was no apparent change in the nature of the waves.

(b) In 2 animals after a fall in the plateau level the waves became deeper and more regular.

(c) In one animal after procaine block of the uterine mesentery the plateau level fell only 3 mm. Hg, but the small waves of pressure which had been observed in the control period disappeared completely. In another animal the plateau level fell from 72 to 48 mm. Hg, and all the waves similarly disappeared. In both of these animals reinsufflation was performed and all the waves reappeared (Fig. 3).

3. Effect upon the tracing gained through reinsufflation. This was performed in 7 animals.

(a) In all cases the primary peak at which gas entered the abdominal cavity was lower in the second insufflation obtained after blocking the uterine mesentery than in the control tracing. The arithmetic mean for the first tracing was 109 mm. Hg and for the second was 62 mm. Hg. This decrease is not to be attributed solely to procaine, for it has been shown that simple reinsufflation of itself lowers the primary pressure peak. (In a group of ten animals who were reinsufflated without procaine injection the mean for the primary peak of the first tracing was 130 mm. Hg and of the second 79 mm. Hg).

(b) In all cases the plateau level of the second insufflation was the same or slightly lower than that obtained under procaine.

(c) The small waves of pressure usually appeared more regularly and were deeper than in the previous insufflation.

4. Saline infiltration of uterine mesentery. In 3 animals saline solution in 1 to 1.5 c.c. amounts was injected into the uterine mesentery to rule out any mechanical effect of fluid distention. In 2 animals no change in the tracing

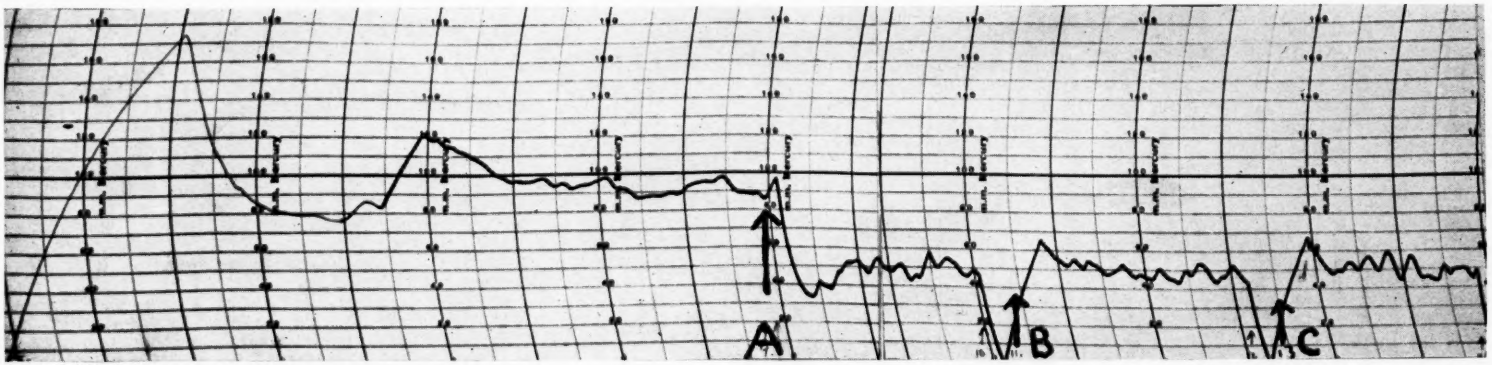

Fig. 1.—Effect of procaine injection of the uterine mesentery. Primary peak—174 mm. Hg. Plateau level—90 mm. Hg. At *A*, injection of 1 c.c. of 2 per cent procaine hydrochloride into the uterine mesentery. *Note:* Immediate drop to level of 45 mm. Hg and maintenance of level upon reinsufflation at *B* and *C*.

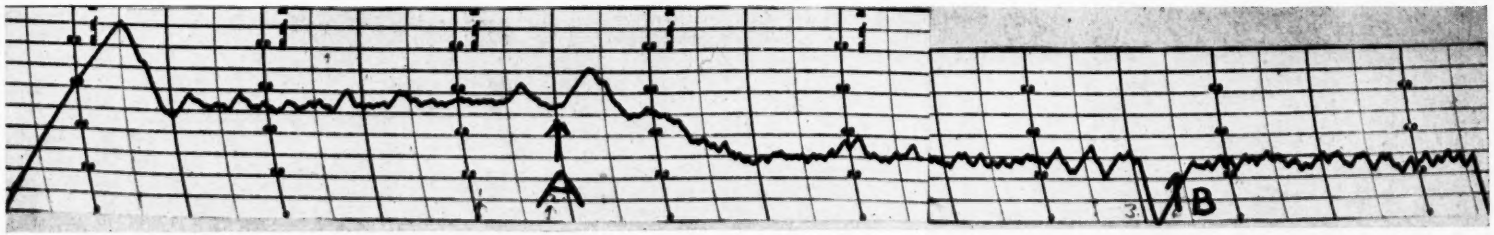

Fig. 2.—Effect of procaine injection of the uterine mesentery. Primary peak—92 mm. Hg. Plateau level—57 mm. Hg. At *A*, injection of 1 c.c. of 2 per cent procaine hydrochloride into the uterine mesentery. *Note:* Drop to level of 28 mm. Hg and maintenance of level upon reinsufflation at *B*.

occurred. In one animal the plateau level fell 7 mm., but the character of the tracing remained the same. In all 3 cases procaine was then injected into the areas previously infiltrated with saline. In 2 of the 3 animals the plateau level was decreased about 15 mm. Hg (there was considerable dilution of the procaine by previously injected saline), and the reinsufflation curves were similar to those seen under procaine insufflation.

5. In one animal no gas entered the cavity, although a pressure of 200 mm. Hg was reached. The animal was reinsufflated and the uterine mesentery injected with procaine while the pressure was still rising. Gas entered to form a primary peak at a level of 175 mm. Hg and fell to a plateau of 70 mm. Hg. Another cubic centimeter of procaine was injected into the mesentery, and the level fell further to 55 mm. Hg. On reinsufflation the primary peak was at a level of 88 mm. Hg and the plateau was 60 mm. Hg.

TABLE II

CONTROL PRIMARY PEAK	PLATEAU	UNDER TUBOOVARIAN BLOCK	UNDER UTERINE BLOCK
mm. Hg	mm. Hg		
108	78	98, fell to 78	64
170	95	135, fell to 60	
124	63	45, reinsufflated to produce a primary of 90 and plateau of 45	30
168	80	Up to 100	45
152	52	Up to 58	To 35 and rose gradually to 55

TABLE III

RATE OF GAS FLOW IN C.C./MIN.	PRIMARY PRESSURE PEAKS IN MM. Hg	
	OVULATING	NONOVULATING
11	140	
15	148, 78	108
18	155, 105, 90	143
19		117
20	158, 127, 54	152, 142
21	200, 50	
22		200, 168, 168, 124, 119, 100
24		200, 100
25		168
27		115
28	128	183
30	74	80, 65
32		174
35	173	92
37		150
40	100	
42		174
50		88

Nerve Block of the Tuboovarian Mesentery.—1. In 2 rabbits 1 c.c. of saline was injected into the tuboovarian mesentery and no change was observed in the tracing.

2. In a rabbit whose uterine mesentery had previously been injected with procaine to produce a slight fall in plateau level, injection of the tuboovarian mesentery produced no change.

3. In 5 experiments procaine was first injected into the tuboovarian mesentery. In 4 of these cases the plateau level rose slightly while in one case it fell. In 4 cases the uterine mesentery was then infiltrated with procaine and a fall was observed in the plateau level in all cases (Figs. 4 and 5).

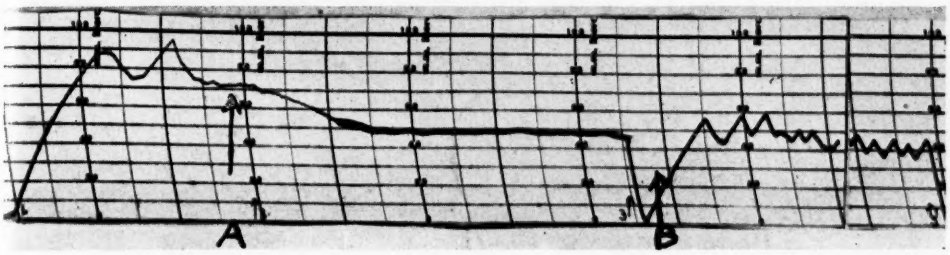

Fig. 3.—Effect of procaine injection of the uterine mesentery. Primary peak—88 mm. Hg. Plateau level—72 mm. Hg. At *A*, injection of 1 c.c. of 2 per cent procaine hydrochloride into the uterine mesentery. *Note:* Drop to level of 48 mm. Hg and disappearance of all waves on the tracing. On reinsufflation at *B*, the waves returned.

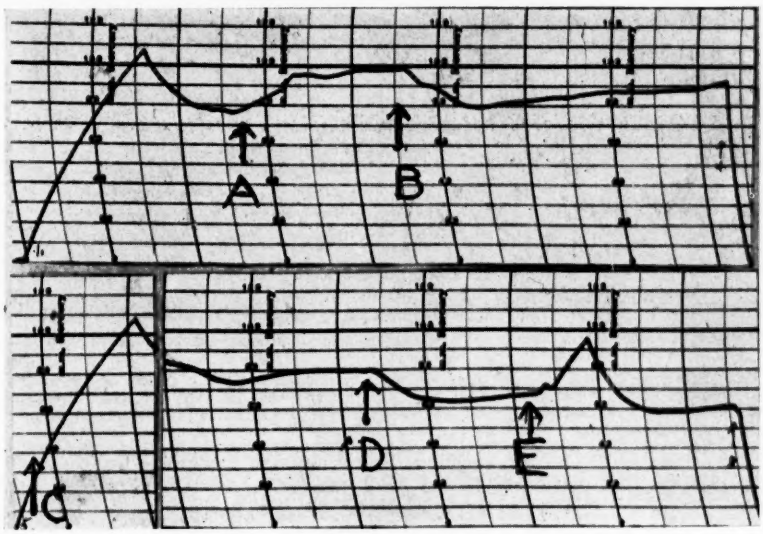

Fig. 4.—Effect of block of the tuboovarian mesentery followed by block of the uterine mesentery. Primary peak—108 mm. Hg. Plateau level—78 mm. Hg. At *A*, injection of 1 c.c. of procaine into the tuboovarian mesentery. At *C*, reinsufflated. At *D*, another cubic centimeter of procaine injected into the uterine mesentery. At *E*, another cubic centimeter of procaine injected into the tuboovarian mesentery. *Note:* Drop in levels on injecting the uterine mesentery and rise in levels upon injecting the tuboovarian mesentery.

Effect of Induced Ovulation.—In 15 animals ovulation was induced by the injection of human pregnancy urine. Ten cubic centimeters of urine were injected two days in succession, and on the third day insufflation was performed. The effect upon the primary pressure peaks was compared to that of 23 nonovulating animals.

The arithmetic means for the two groups are 104 for the ovulating animals and 136 for the nonovulating. This difference is insignificant in view of the scattering.

DISCUSSION

We have demonstrated that definite changes are observed in the kymographic tracings after blocking the uterine mesentery with a local anesthetic.

The actual part played by the ovarian plexus as compared to the uterine, as well as the factors of the sympathetic as opposed to parasympathetic innervation, have not as yet been elucidated.

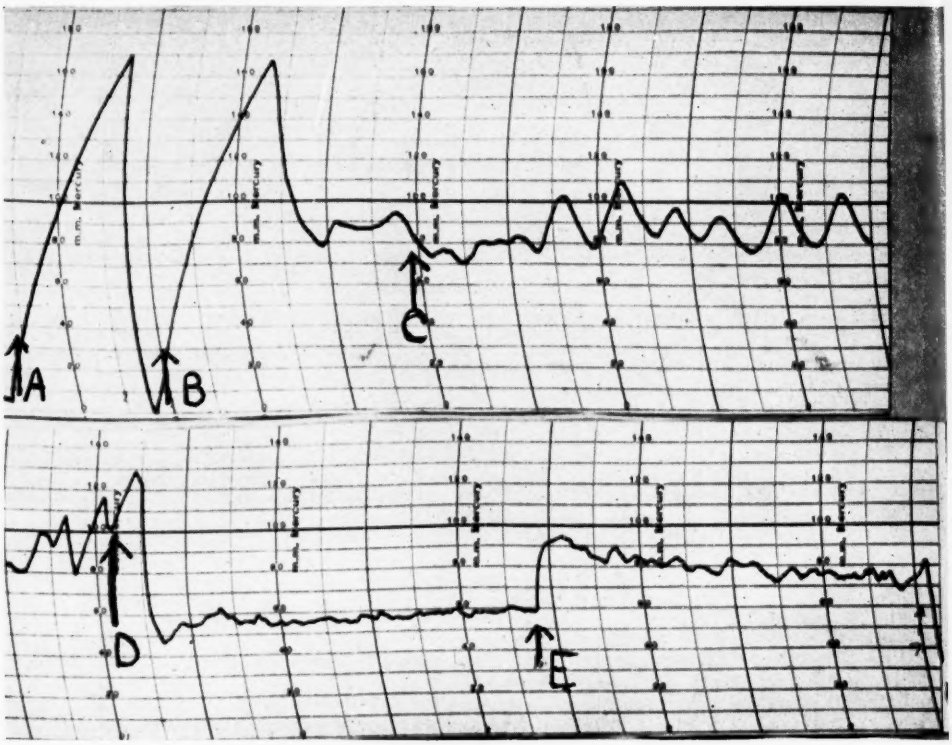

Fig. 5.—Effect of block of tuboovarian mesentery followed by block of uterine mesentery. The animal was insufflated at *A* with no gas passing into abdominal cavity. Reinsufflated at *B* at the same rate of gas flow. Primary peak—168 mm. Hg. Plateau level—80 mm. Hg. At *C*, 1 c.c. of procaine injected into the tuboovarian mesentery. At *D*, 1 c.c. of procaine injected into the uterine mesentery. At *E*, the rate of gas delivery was increased from 20 to 100 c.c. per minute. *Note:* Rise in level after injecting the tuboovarian mesentery and fall in level after injecting the uterine mesentery. On speeding the rate of gas delivery five times, the level rose 35 mm. Hg and gradually fell.

In a previous communication it was demonstrated that upon simple re-insufflation of rabbits both the primary peaks and the plateau levels fall to reach a constant level after about thirty minutes of insufflation. This particular level is promptly reached after the injection of procaine into the uterine mesentery. Furthermore, although an increase in gas rate increases the primary peak and the plateau levels,[1] upon continuing the insufflation the level falls to reach that obtained at slower rates of flow. This phenomenon has been observed in animals whose uterine mesentery has been blocked. Thus there seems to be a basic level of insufflation pressure, or plateau level, which is kept fairly constant by some intrinsic compensating mechanism, irrespective of time, nerve impulses, and delivery rate.

The obvious clinical applications of these findings are now being studied on patients, with particular reference to the case management of sterility and the relief of dysmenorrhea.

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NONFILAMENT-FILAMENT RATIO CHANGES FOLLOWING ADMINISTRATION OF AUTOGENOUS VACCINES TO PERSONS WITH LOW-GRADE CHRONIC ILLNESS

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IT HAS been stated that the ratio of immature to mature neutrophilic leucocytes parallels the activity of an infectious disease process[1] and the severity of symptoms,[2] and that clinical improvement in patients with low-grade chronic illness is accompanied by proportionate improvement in the immature-mature cell ratio.[3] It has been suggested also that the ratio of immature to mature neutrophiles might be used in the control of vaccine therapy.[1a, 1e] The present study was made to determine whether there might be changes in the ratio of young to old neutrophiles which would correlate with the subjective responses which sometimes follow the injection of autogenous vaccines.

MATERIAL AND METHODS

Observations were made on a group of persons with low-grade chronic illness in whom chronic infection was considered to be an important factor. Detailed analyses of this group as to clinical condition, sedimentation rate, and nonfilament-filament ratio,[2] and incidence of probable pathogenic streptococci and staphylococci[4] have been reported. Autogenous vaccines prepared from organisms isolated and selected by methods previously described[4, 5] were administered subcutaneously in varying dilutions to these persons. At times following injection of the diluted vaccines there were definite subjective responses which could be reproduced by reinjection of the same doses of vaccine at appropriate intervals.

Blood smears were made at frequent intervals, sometimes several times daily when the sequence of changes was rapid, from persons in whom definite subjective responses were noted. Differential blood counts were made, neutrophiles being divided into nonfilamented and filamented types (or nonsegmented and segmented types, no distinction being made between the two methods[6]), according to the criterion of Haden,[7] i.e., "neutrophiles in which two or more lobes are united only by a filament of chormatin material are recorded as filamented cells, all others are classified as nonfilamented." The ratio of nonfilamented to filamented neutrophiles (referred to as "nonfilament-filament ratio") was expressed in percentage; i.e., nonfilament-filament ratio $= \frac{\text{nonfilamented cells} \times 100}{\text{filamented cells}}$.[2]

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RESULTS

To a large extent the changes in the nonfilament-filament ratio paralleled subjective changes, i.e., when symptoms were increased the nonfilament-filament ratio was increased, and when symptoms were lessened the nonfilament-filament ratio was lessened.

Three general types of subjective response to vaccine injection were noted, with corresponding nonfilament-filament ratio changes:

Type I: In the first type there was a period of definite lessening or cessation of symptoms, accompanied by a feeling of well-being, lasting from a few days to a week or more.

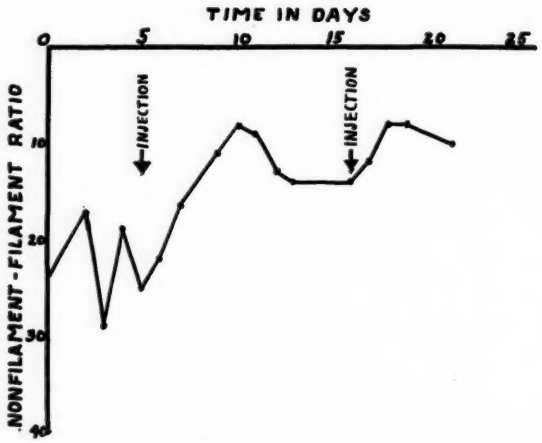

Fig. 1.—Variations in the nonfilament-filament ratio during a five-day control period and after two injections of vaccine which were followed by subjective improvement (type I response).

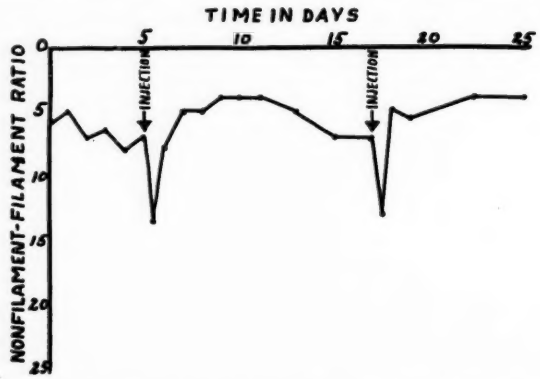

Fig. 2.—Variations in the nonfilament-filament ratio during a control period and after vaccine injections in a person who developed transient unfavorable symptoms (less than twenty-four hours) followed by definite improvement (type II response).

Type II: The second type of response was characterized by an initial period of increased intensity of illness, with malaise, depression, headache, or other general symptoms (the so-called negative phase), followed by a period of definite improvement. The negative phase lasted from a few hours to a few days, and the subsequent period of improvement from a few days to a week or more.

Type III: The third type was characterized by a prolonged negative phase, lasting a week or more, with little or no subsequent improvement. In this type

the unfavorable symptoms of the negative phase were usually milder in intensity and more delayed in onset than the unfavorable symptoms in type II responses.

In Figs. 1, 2, and 3 are shown typical examples of the nonfilament-filament ratio changes accompanying the different types of subjective response. In each instance there are included observations of the nonfilament-filament ratio made on each of the five days prior to the injection of vaccine, and the observations made subsequent to two injections.

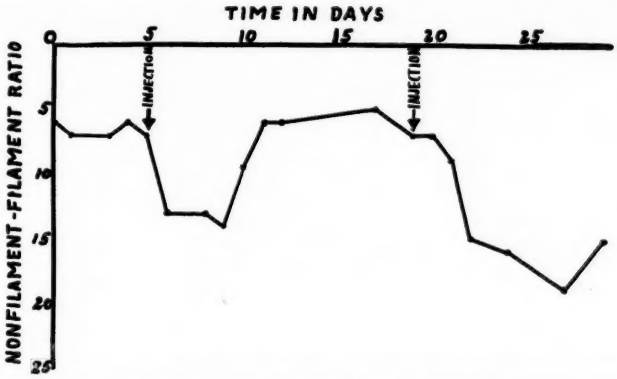

Fig. 3.—Variations in the nonfilament-filament ratio during a control period and following vaccine injections in a person who developed prolonged unfavorable reactions with very little subsequent improvement (type III response). Note that following the second injection there was a delay of more than a day in the onset of the unfavorable change in the nonfilament-filament ratio.

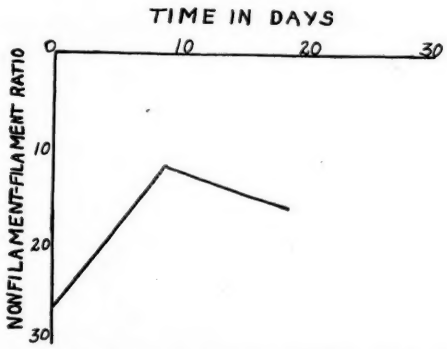

Fig. 4.—Composite graph based on the means of the nonfilament-filament ratio observations in 28 persons who improved following vaccine injection (type I response).

Fig. 4 is a composite graph based on the means of the nonfilament-filament ratio observations at the time of injection, at the time of greatest improvement, and at the time of final observation, of 28 persons who improved following vaccine injection (type I response). The mean nonfilament-filament ratios were 26.75, 11.6, and 16.0, respectively. The separate graphs of the 28 persons are shown in Figs. 4*A*, 4*B*, and 4*C*.

Fig. 5 is a composite graph similarly based on the means of the nonfilament-filament ratio observations of 12 persons who experienced preliminary unfavorable symptoms following injection. In these persons the unfavorable reaction, which lasted from one to five days, was followed by a period of definite improvement (type II response). The mean of the nonfilament-filament ratios at the time of injection was 18.4; at the time of greatest increase in symptoms, 30.0;

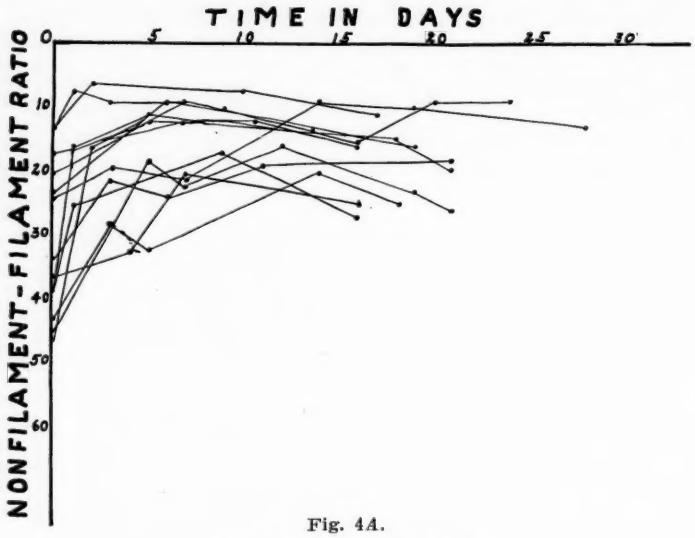

Fig. 4*A*.

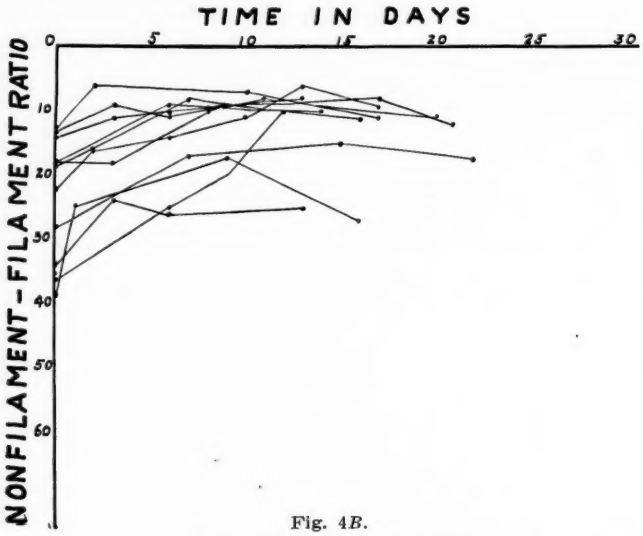

Fig. 4*B*.

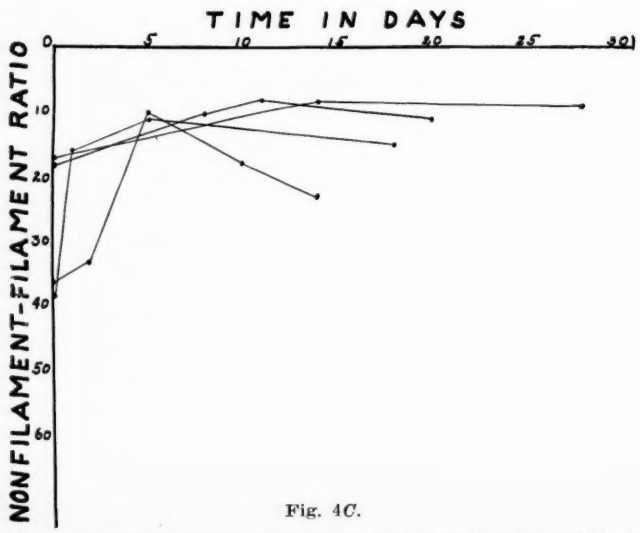

Fig. 4*C*.

Figs. 4*A*, 4*B*, and 4*C*.—Separate records of nonfilament-filament ratios used in computing Fig. 4.

at the time of greatest improvement, 10.5; and at the time of final observation, 14.3. The separate curves of nonfilament-filament ratio change in the 12 persons are shown in Fig. 5A.

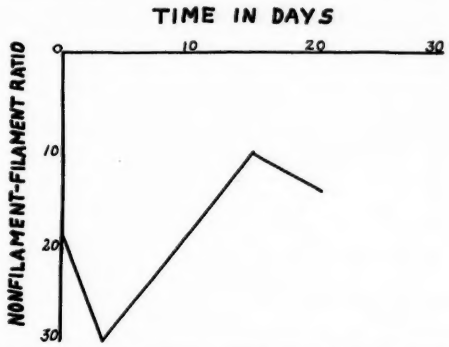

Fig. 5.—Composite graph based on the means of the nonfilament-filament ratio observations in 12 persons who, after vaccine injection, experienced preliminary unfavorable symptoms followed by definite improvement (type II response).

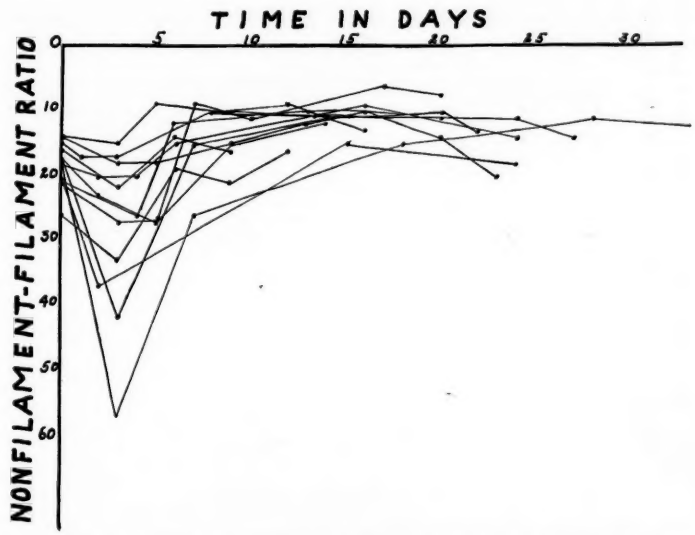

Fig. 5A.—Separate records of nonfilament-filament ratios used in computing Fig. 5.

Fig. 6 is a composite graph based on the means of the nonfilament-filament ratio observations of 8 persons in whom there was a prolonged unfavorable reaction (type III response). The mean of the individual nonfilament-filament ratios at the time of injection was 15.5; at the time of the most severe symptoms, 29.3; at the time of greatest improvement, 13.0; and at the time of final observation, 14.9. The separate graphs of the nonfilament-filament ratio changes of the 8 persons are shown in Fig. 6A. In this group of persons there was little, if any, subjective improvement following the prolonged unfavorable reaction, and the mean of the nonfilament-filament ratio observations at the time of greatest improvement was very little better than the mean of the original observations.

Providing there was no intercurrent illness, there were no significant nonfilament-filament ratio variations observed in persons receiving no injections,

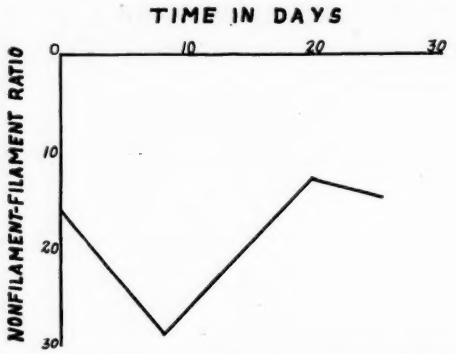

Fig. 6.—Composite graph representing the means of the nonfilament-filament ratio observations in 8 persons who experienced prolonged unfavorable reactions with little or no subsequent improvement (type III response) following vaccine injection.

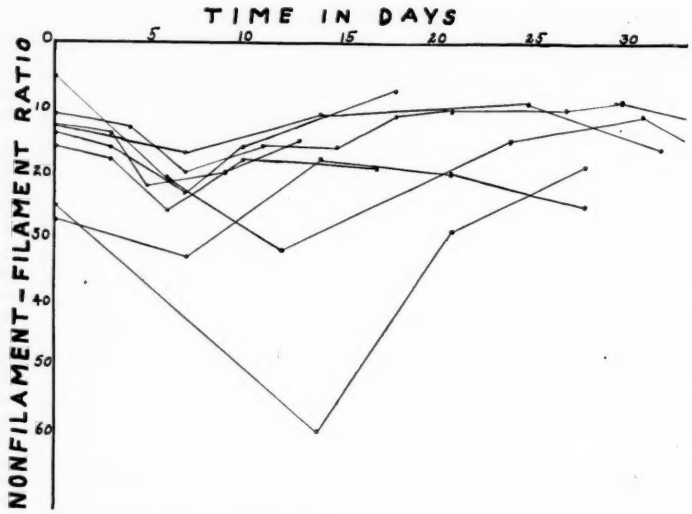

Fig. 6A.—Separate records of nonfilament-filament ratios used in computing Fig. 6.

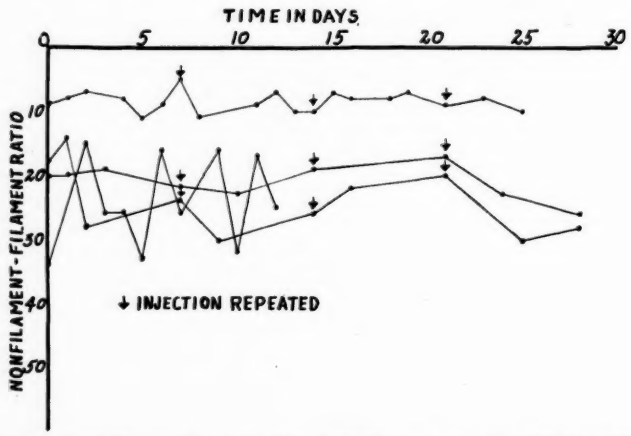

Fig. 7.—Nonfilament-filament ratios after injections of vaccine diluting fluid (0.5 per cent phenol) in 4 persons. Injections were made at the time of initial observation and were repeated as indicated.

e.g., during initial five-day periods (Figs. 1, 2, and 3), or in persons receiving injections either of diluting fluid (Fig. 7), or of vaccine too dilute to produce any subjective response (Fig. 8).

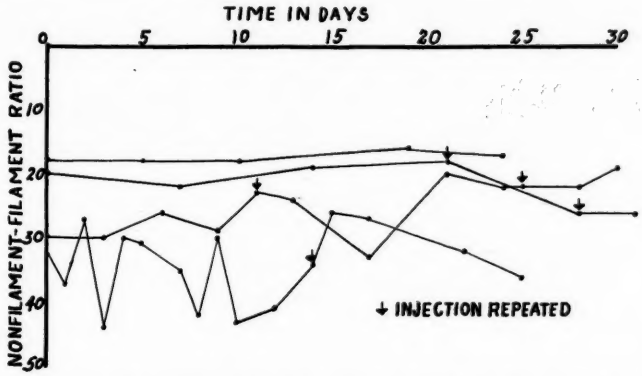

Fig. 8.—Nonfilament-filament ratios of 4 persons in whom there was no evidence of subjective response following the injection of excessively diluted vaccine. (In Fig. 4*C* are shown the nonfilament-filament ratios of the same persons following the injection of stronger vaccines which produced type I responses.)

TABLE I

STATISTICAL ANALYSIS OF NONFILAMENT-FILAMENT RATIO CHANGES FOLLOWING THE INJECTION OF AUTOGENOUS VACCINES*

	NONFILAMENT-FILAMENT RATIO			
	NO.	MEAN	S.D.	S.E.
Type I response				
Observations at time of injection	28	26.75	11.23	2.12
Observations during greatest improvement	28	11.57	4.60	0.87
Final observations	28	16.04	6.32	1.19
Type II response				
Observations at time of injection	12	18.42	4.31	1.30
Observations during most severe symptoms	12	30.08	12.29	3.72
Observations during greatest improvement	12	10.05	4.01	1.21
Final observations	12	14.25	2.97	0.895
Type III response				
Observations at time of injection	8	15.50	7.07	2.67
Observations during most severe symptoms	8	29.25	12.18	4.61
Observations during greatest improvement	8	13.00	4.34	1.64
Final observations	8	14.88	4.29	1.62

*No. indicates number of cases; S.D., standard deviation; S.E., standard error.

COMMENT

A statistical analysis of the nonfilament-filament ratio observations is presented in Table I. The differences between the means of the initial observations and the means of the observations at the point of most unfavorable symptoms were statistically significant (5.2 times the standard error in type II responses and 5.1 times the standard error in type III responses). Significant also were the differences between the means of the observations at the point of most unfavorable symptoms and at the point of greatest improvement (8.8 times the standard error in type II responses and 6.5 times the standard error in type III responses). The differences between the means of the initial observations and the means of the observations at the point of greatest improvement were statistically significant except in patients who experienced prolonged unfavorable

reactions (8.2 times the standard error in type I responses and 4.5 times the standard error in type II responses, but only 1.2 times the standard error in type III responses).

While in general the changes in the nonfilament-filament ratio paralleled the subjective changes, there were a few exceptions, probably because in these instances the symptoms might have been influenced by emotional, climatic, allergic, or other factors without corresponding changes in the leucocyte ratio.

SUMMARY

Nonfilament-filament counts were made at frequent intervals on persons showing definite subjective response to injections of autogenous vaccines. When there was an increase in symptoms, there was an increase in the nonfilament-filament ratio. When there was a lessening of symptoms, there was a lessening of the nonfilament-filament ratio. The variations in the nonfilament-filament ratio were statistically significant.

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STUDIES ON ANTIPNEUMOCOCCIC RABBIT SERUM*

I. The Correlation Between the Thermal Reaction Test in the Rabbit and the Incidence of Clinical Reactions in Pneumonia Patients

Tillman D. Gerlough, B.S., Reuben R. Blumenthal, Ph.D., and John W. Palmer, Ph.D., New Brunswick, N. J.

SABIN and Wallace[1] described a test for the presence of chill-producing substances in concentrated antipneumococcic horse serum in which dogs were injected intravenously with the serum. A rise in rectal temperature of 1.5° F. or more in about an hour indicated sufficient chill-producing material to cause reactions in patients treated with the serum. Barnes and Robinson,[2] using monkeys in a similar test, did not obtain as good agreement between their laboratory and clinical results.

Goodner, Horsfall, and Dubos[3] have correlated thermal reactions produced in rabbits with incidence and severity of chill or febrile reactions in pneumonia patients when both were injected intravenously with the same antipneumococcic rabbit serum. They state that if the mean rise in temperature of a group of rabbits, each of which receives 2.0 ml. of serum, exceeds 1.2° F., the serum will produce chills in patients, and if the mean rabbit thermal reaction is over 2.0° F., the serum will produce such severe chills that it should not be used clinically. In their experience sera producing a thermal rise in rabbits of less than 1.2° F. gave no chills in patients. It was emphasized that the correlation was established only for sera prepared and processed by their procedure. Their sera were not concentrated but were treated with heat and kaolin.

It was desirable to know whether or not any such relationship existed in the case of concentrated antipneumococcic rabbit serum. During the development of refining and concentrating procedures for rabbit serum, more than 20 lots of concentrated serum were subjected to both clinical and rabbit tests, and a good degree of correlation was found.

Table I records the data. The rabbits employed for thermal tests were selected as follows: On the day of the test the rectal temperatures were taken. Animals with a normal temperature greater than 103.6° F. were not used. One milliliter of a freshly prepared pyrogen-free 0.9 per cent sodium chloride solution containing 0.2 per cent phenol and 0.01 per cent merthiolate was then injected intravenously, and the rectal temperature was taken at the end of one hour. If at this time the temperature had not varied more than 0.6° F. from its normal, the animal was then used for the thermal test of the serum.

*From the Biological Laboratories of E. R. Squibb & Sons, New Brunswick.
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Routinely 1.0 ml. of the concentrated serum was injected into an ear vein of a rabbit in one minute. (This dose is of the same order as that employed by Goodner, Horsfall, and Dubos with respect to the amount of protein administered.) The temperature was read one hour and two hours after the injection. The temperature difference at the one-hour interval was taken as the most significant, and has been used in the tabulations.

TABLE I

CLINICAL AND LABORATORY OBSERVATIONS ON CONCENTRATED ANTIPNEUMOCOCCIC RABBIT SERUM

CONCENTRATED SERUM			NUMBER OF PATIENTS		% INCIDENCE OF CHILL REACTIONS		TEMPERATURE RISE IN RABBITS (° F.) ± P.E.†	NUMBER OF RABBITS
TYPE	LOT	POTENCY (UNITS PER Ml.)	TREATED	SHOWING CHILL REACTION	OBSERVED	CALCULATED*		
I	1	10,000	65	2	3.1	3.6	0.20 ± 0.07	5
	2	10,000	9	3	33.3	28.1	0.87 ± 0.14	6
	3	12,000	19	9	47.4	32.5	0.94 ± 0.11	8
	4	10,200	4	1	25.0	6.6	0.36 ± 0.05	18
II	1	4,500	3	3	100.0	85.1	1.76 ± 0.10	24
	2	7,250	7	1	14.3	6.3	0.35 ± 0.09	13
	3	7,200	5	0	0.0	2.7	0.13 ± 0.06	14
III	1	2,500	14	11	78.6	82.9	1.71 ± 0.10	19
	2	9,450	11	2	18.2	25.1	0.82 ± 0.06	25
	3	16,300	9	1	11.1	11.2	0.52 ± 0.07	14
	4	14,500	3	1	33.3	8.1	0.42 ± 0.09	14
IV	1	8,400	6	2	33.3	55.2	1.26 ± 0.08	18
	2	10,950	3	1	33.3	3.4	0.19 ± 0.04	14
V	1	8,000	7	4	57.1	67.7	1.44 ± 0.13	8
	2	11,330	2	1	50.0	7.3	0.39 ± 0.06	20
VII	1	17,000	4	3	75.0	73.0	1.54 ± 0.13	8
	2	16,200	5	0	0.0	6.1	0.34 ± 0.05	14
	3	18,000	7	2	28.6	14.6	0.61 ± 0.06	22
VIII	1	7,870	5	1	20.0	31.8	0.93 ± 0.06	8
	2	16,700	3	0	0.0	5.7	0.32 ± 0.07	6
XIV	1	5,900‡	2	0	0.0	3.3	0.18 ± 0.02	14

*See text.

†Probable error $= 0.6745\ \sigma/\sqrt{n}$, where σ is the standard deviation and n is the number of rabbits used to test the lot of serum.

‡Calculated from antibody nitrogen.[4]

Five to 8 rabbits were used for each routine test. In many instances the test was carried out on the concentrates before and after sterile filtration. No significant differences were found in the two samples. All samples tested prior to sterile filtration were passed through a small Seitz E.K.S-3 asbestos pad to remove dust, lint, and other insoluble matter.

The potency of the antisera for types I, II, V, VII, and VIII pneumococci was determined by comparison in mouse protection tests with the standard sera distributed by the National Institute of Health. The sera for types III and IV were standardized in terms of Felton mouse protective units as determined in our laboratories, there being no official standard sera for these types. The

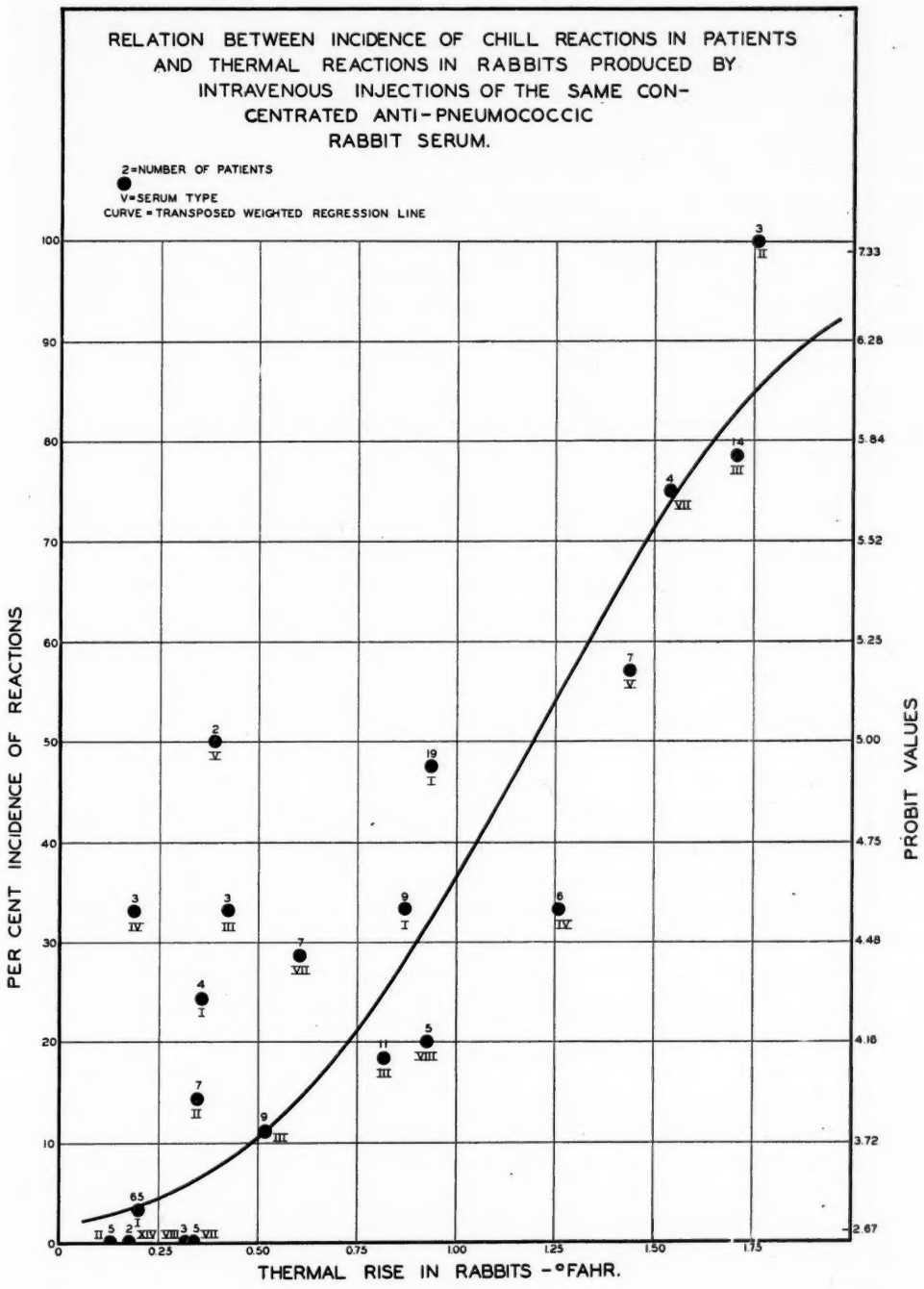

Fig. 1.

type XIV antiserum was evaluated by the quantitative precipitin reaction,[4] 1 mg. of specifically precipitable antibody nitrogen representing 750 units, as recommended by the National Institute of Health.[5]

The clinical data are taken from reports received from 24 physicians located in various parts of the country.

Inspection of Table I reveals, for most lots, a definite relationship between the laboratory rabbit test and clinical observation. There is, of course, some dispersion which is particularly evident with lots for which only a few clinical reports were received.

The reports show that an average of 170,000 units of antibody was administered to each patient. In general, the chill or febrile reaction, when produced, occurred after the first large dose of about 50,000 units. Subsequent injections of the same serum were, for the most part, uneventful. Chills were usually severe if produced by a concentrated serum which caused a rise of 1.5° F. or more in rabbits. Potency or type of serum apparently had no significance as far as reactions were concerned.

It is not known what substance or substances in rabbit serum cause the thermal or chill reaction. In the statistical treatment of the data, it was assumed that the reactions were due to the same general type of pyrogenic substance which varied in amount from lot to lot. The thermal rise in rabbits was taken as a measure of the concentration or dose of pyrogenic material, and the incidence* of chill reactions in patients as an expression of the effect. This dose-effect reactionship probably follows a typical sigmoid curve.

The data were plotted on arithmetic probability paper, and the percentage incidence of chill reactions was converted into probits.[6] The weighted correlation coefficient† between the thermal rise in rabbits (in degrees Fahrenheit) and the probit values of percentage incidence of reactions in patients is 0.84, based on 193 patients and 292 rabbits treated with 21 lots of serum. In view of this high degree of correlation, a prediction equation was calculated. This equation is

$$I = 1.821T + 2.835,$$

where I is the probit value of the percentage incidence and T is the thermal rise in rabbits. Column 7 of Table I shows the value for incidence of chills as predicted by the above equation. The weighted average percentage deviation† of the observed from the calculated values is ± 7.0 per cent of the calculated values.

The sigmoid curve in Fig. 1 is a calculated weighted curve transposed from the regression line obtained on arithmetic probability paper, and is, therefore, a graphic expression of the prediction equation given above.

SUMMARY

A high degree of correlation (0.84) has been found between the temperature rise produced in rabbits and the incidence of chill reactions in patients with pneumonia following the administration of concentrated antipneumococcic rabbit serum.

An equation was developed permitting the prediction of incidence of chill reactions, the weighted average deviation of the calculated from the observed values being ± 7.0 per cent.

We wish to express our appreciation to Mr. H. A. Holaday and his associates of the Biological Assays Laboratories for carrying out the thermal tests in rabbits, and to Dr. G. F. Leonard and Dr. August Holm of the Bacteriological Laboratories for the mouse protection potency tests.

*Incidence is defined here as the percentage of patients treated with a given serum who developed a chill or chills. In general, the chill reaction was accompanied by a rise in temperature.

†Weights were assigned each observation in proportion to the number of patients treated with each lot of serum.

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SPECIFIC CHEMOTHERAPY OF GIARDIA INFECTIONS*

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ALTHOUGH many widely different chemotherapeutic agents had been tried previously in the therapy of giardiasis, no effective drug was available until recent years for the permanent elimination of the giardias from man and animals. In 1937 Brumpt,[1] working with mice infected with *Giardia muris*, found that the administration by mouth of a 1 per cent solution of "quinacrine" (the French equivalent of atabrine) permanently cured 80 per cent of the infections. Later in the same year, Galli-Valerio[2] reported the successful elimination of *Giardia lamblia* from man by the administration of atabrine. A number of papers[3] have since confirmed these first observations, and atabrine seems now fairly well established as an effective and safe drug for the treatment of giardiasis. The present communication offers further evidence upon the action of atabrine upon giardia infections of man, and of the albino rat and mouse.† The paper reports also that acriflavine, another acridine derivative, likewise is an effective drug in giardia infections of animals.

ATABRINE IN GIARDIA INFECTION OF MAN

Two cases of *Giardia lamblia* infection of man have been treated with atabrine. The first of these concerned an individual in whom the extraordinary persistence of the parasite has previously been reported by Miles and myself.[4] Because this case responded so promptly to atabrine therapy, permanent cure being effected by a single course of treatment, the use of the drug in it will be described in some detail. The second human case, of which much less is known, will be only briefly mentioned.

Cure by Atabrine of a Long-Persisting Giardia Infection in Man.—The patient was a young woman of about 25 years of age, whose infection had been first

*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

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†The atabrine used in the work was supplied by the Winthrop Chemical Co., New York, N. Y.

diagnosed approximately six years earlier. At irregular intervals since the initial observation, the parasites or their cysts had been found in the patient's stools, search being made at least once each year and during some years frequently. An ineffectual attempt was made in the summer of 1936 by an attendant physician to eliminate the parasites by administering carbarsone. Cysts of the parasite were believed reduced in number thereafter for a time, but they never entirely disappeared. Soon after the cessation of treatment, they became as numerous as they had been originally.

Cysts of the parasite were observed in large number in January, 1940, one day prior to the institution of treatment with atabrine. Atabrine treatment was begun under the direction of Dr. C. W. Jungeblut of this department, two 0.1 Gm. tablets being given daily for five days. A stool specimen obtained two days after the last day of treatment was negative for cysts and trophozoites of *Giardia lamblia*, prolonged search being made of repeated samples of stool. Likewise, when stools obtained one, two, four, and six weeks later were examined, no cysts or trophozoites of *Giardia lamblia* could be found.

No significant by-effects of treating the patient with atabrine were noted. On the last day of treatment the patient felt listless and sneezed occasionally, but these symptoms were not certainly related to the use of the drug. No pigmentation of the skin was observed at any time. One day after the cessation of treatment, the stool was said by the patient to be somewhat watery and of a bright yellow hue, but subsequent stools were normal.

Cure by Atabrine of a Second Case of Human Giardiasis.—A second case of giardiasis in man was also treated successfully with atabrine, the drug being administered by Dr. H. M. Rose of this department. A stool examined just prior to the institution of treatment revealed many cysts of *Giardia lamblia*. Two 0.1 Gm. tablets of atabrine were given daily for five days. Stools obtained for three successive days after the last day of treatment were examined carefully, but neither cysts nor trophozoites of the parasite were seen. This patient has not since returned for observation.

ATABRINE IN GIARDIA INFECTION OF ALBINO RATS

Thirty rats naturally infected with *Giardia muris* were used to test the therapeutic action of atabrine upon giardiasis of rats, the infection being proved to exist in each rat by finding cysts of the parasite in a saline suspension of feces of the animal. All rats were small when used, weighing about 30 Gm. Atabrine in water solution was administered by mouth to 20 of the infected animals on two successive days, each dose being 10 mg. per 30 Gm. body weight. The drug was given by syringe through a blunted hypodermic needle (size 22; 1.5 inch length) lowered carefully into the pharynx or esophagus. The 10 remaining infected rats received no drug at any time, and were kept as controls of the infection in cages separate from the treated rats. The feces of the treated and control animals were examined daily for one week after the last dose of the drug. All the treated animals were free of the parasite, so far as fecal examination revealed by the first day after treatment, and remained so during the ensuing week (Table I). The 10 control rats retained their infections throughout this

period. Twelve of the treated rats were kept for three additional weeks, and their feces were examined at weekly intervals during this period. No animal revealed giardia cysts at any examination. At the end of this time these 12 rats were autopsied, and the small intestine was searched for trophozoites of *Giardia muris*. None was seen in any animal. It appears from this work that *Giardia muris* is promptly removed from the intestine of albino rats by the administration of 10 mg. of atabrine per 30 Gm. of rat body weight daily for two days, and that infections so treated do not relapse, at least during one month following treatment.

TABLE I

EFFECT OF ATABRINE AND OF ACRIFLAVINE UPON GIARDIA INFECTIONS IN DIFFERENT HOSTS

GIARDIA SPECIES	HOST SPECIES	DRUG USED	TOTAL DOSE OF DRUG GIVEN	NO. HOSTS TREATED	NO. HOSTS CURED	PERIOD OF OBSERVATION AFTER TREATMENT	UNTREATED CONTROLS	
							NO. USED	NO. POSITIVE FOR AT LEAST ONE WEEK
Giardia lamblia	Man	Atabrine	1 Gm.	2	2	3 days; 6 weeks	None*	–
Giardia muris	Rat	Atabrine	20 mg.†	20	20	1 week to 1 month	10	10
Giardia muris	Mouse	Atabrine	20 mg.†	10	10	1 week	5	5
Giardia muris	Rat	Acriflavine	20 mg.†	10	10	1 week	5	5

*One of the human cases was positive for over five years prior to treatment.
†Per 30 Gm. body weight.

It should be added that the rats tolerated the atabrine very well, and no animal was lost during the course of the experiment. The dose was, however, large in proportion to that given to the patients described earlier, and all the treated animals presented yellow pigmentation of the skin on the day after the second dose of drug was given. The pigment persisted for one week, and in some cases for longer periods. In several other rats given this dose of atabrine and autopsied one or two days thereafter, the small intestine was found deeply stained with the color of atabrine, particularly in those parts where *Giardia muris* is most commonly found.

ATABRINE IN GIARDIA INFECTION OF ALBINO MICE

Fifteen mice, all known from fecal pellet examination to harbor *Giardia muris*, were used to test the effect of atabrine upon mouse giardiasis. Ten animals were given 10 mg. of atabrine by mouth per 30 Gm. body weight on each of two successive days in the same manner as previously described for the infected rats. The remaining 5 mice were kept as controls for the natural persistence of the infection in the absence of treatment. The feces of all animals were examined daily for the presence of cysts of the parasite for one week, beginning one day after the last dose of drug. All treated mice were free of the infection, so far as fecal examination revealed, by the first day after treatment, and remained so throughout the period of observation (Table I). Autopsy at the end of this interval revealed no trophozoites of the parasite in the small intestine. The control mice showed cysts of the parasite in the feces at each of the daily observations until autopsy and then revealed the trophozoite stage in

the intestine. Apparently, cure of giardia infection by the administration of atabrine is about as successful in the mouse as it is in the rat or in man.

ACRIFLAVINE IN GIARDIA INFECTIONS IN ALBINO RATS

The remarkable effect obtained with atabrine upon the different giardiases led to the trial of other acridine derivatives. One of these, acriflavine, a simpler substance than atabrine,* was also found to act powerfully upon giardiasis in the albino rat. This experiment was performed upon 15 albino rats, all proved by fecal examination to harbor *Giardia muris* prior to treatment. The same dose of acriflavine as that used with atabrine (10 mg. per 30 Gm. of body weight on two successive days) was given by mouth to 10 animals, the remaining 5 rats serving as controls. On the first day after treatment and throughout the week following, fecal examination revealed no cysts of *Giardia muris* in the treated animals, although the control rats continued to harbor the parasite throughout this period (Table I). At autopsy one week after treatment, no trophozoites of the parasite were found in the small intestine of the 10 treated animals. From this work it appears that acriflavine is an effective giardiacide. It should be pointed out, however, that the drug in the dose used appeared to harm the rats, for, although none died, all lost weight.

TABLE II

EFFECT OF ATABRINE AND ACRIFLAVINE ON VARIOUS INTESTINAL PROTOZOA OF RATS

DRUG USED IN TREATMENT	NO. RATS IN GROUP	PERCENTAGE OF RATS HARBORING DESIGNATED PROTOZOANS AT AUTOPSY					
		Giardia muris	*Hexamita muris*	*Chilomastix bettencourti*	*Trichomonas muris*	*Trichomonas parva*	*Endamoeba muris*
Atabrine	15	0	14	33	53	67	28
Acriflavine	11	0	9	72	45	72	27
None	23	21	30	65	60	73	56

Dose of drug: 10 mg. per 30 Gm. body weight daily for two days.
Autopsy was performed two days after last dose of drug.

EFFECT OF ATABRINE AND OF ACRIFLAVINE UPON CERTAIN INTESTINAL PROTOZOA OTHER THAN GIARDIA MURIS IN ALBINO RATS

An experiment was designed to test the effect of atabrine and of acriflavine upon certain intestinal protozoa other than *Giardia muris* found in albino rats. For this work, 49 rats of five different litters were used. The feces of these animals were not examined prior to treatment, but part of each litter was kept untreated as control. Fifteen rats were given atabrine and 11 were given acriflavine in the same dosage previously described (10 mg. per 30 Gm. body weight on two successive days); 23 rats received no drug treatment. Two days after the last dose of the drugs, all treated rats and controls were killed and their intestine searched for *Trichomonas muris, Trichomonas parva, Chilomastix bettencourti, Hexamita muris,* and *Endamoeba muris,* as well as for *Giardia muris.* In the case of none of the forms except *Giardia muris* was evidence forthcoming that either atabrine or acriflavine was regularly effective as a destructive agent, although in some instances the relative intensity of infection

*Atabrine: 2-methoxy 6-chloro 9-diethyl amino pentylamino acridine.
Acriflavine: 3, 6-diamino-acridinium-methyl chloride.

in the treated animals appeared somewhat less than in the control rats (see Table II). Of special interest was the persistence of *Hexamita muris* in the treated rats, since this parasite is closely related with *Giardia muris*. This experiment indicated that of the several species of protozoans found in the intestine of the albino rat atabrine and acriflavine exhibit a selective action upon giardia alone.

DISCUSSION

It is obvious from the data given in this paper, as well as from that of a number of other investigators who have previously studied the same problem, that atabrine is an effective drug for the cure of giardia infections of man and animals. Apparently, the giardias can now be placed with a slowly but constantly increasing list of parasites which are subject to control and elimination through the administration of specific chemical substances to the infected individual.

Atabrine appears to be a more favorable drug than acriflavine to use in the elimination of the giardias, judging from the experiments with *Giardia muris* of the rat, for rats treated with an equivalent dose of acriflavine lost weight and usually contracted respiratory infections, whereas those given atabrine showed none of these effects. Nevertheless, acriflavine was as effective as atabrine so far as the elimination of the parasite from the host was concerned.

The discovery of a drug which effectively cures giardia infections in man should render more soluble the old problem of the pathogenicity of *Giardia lamblia* in man. If the parasite is eliminated by atabrine treatment, then symptoms resulting directly from giardia infection should promptly disappear after treatment. On the other hand, if *Giardia lamblia* is not the cause of such symptoms, elimination of the parasite by atabrine cannot be expected to modify these symptoms. In one of the human cases reported in this paper, which was followed for a long period both before and after atabrine treatment, symptoms (epigastric pain, intestinal upset) were unchanged by the drug treatment. Evidently, the presence of the giardias was not responsible for the symptoms in this human case.

SUMMARY

Giardia parasites can be easily eliminated from man, and from albino rats and mice by the administration of atabrine. Apparently the infections are permanently cured by the drug, for relapses following treatment have not been observed in two human infections, one of which was carefully observed during six weeks thereafter, or in infections of laboratory animals which were followed for shorter periods. Reappearance of giardia parasites after treatment would seem, therefore, to occur only as the result of an exogenous reinfection contracted after the force of the drug were spent. The cure of the infection is rapid, no giardia cysts being seen even as early as the first day following the last dose of the drug in patients or in laboratory animals. Of especial interest is the fact that a single course of treatment sufficed to cure, presumably permanently, one of the human cases which had persisted previously for over five years.

Acriflavine, another acridine derivative, likewise will cause the disappearance of *Giardia muris* from the albino rat, although this drug seems less well tolerated by the animal than atabrine.

Neither atabrine nor acriflavine in the dose given to cure giardia infections is effective in eliminating infections with *Trichomonas muris, Trichomonas parva, Chilomastix bettencourti, Hexamita muris,* or *Endamoeba muris* from the albino rat.

I wish to thank Dr. C. W. Jungeblut and Dr. H. M. Rose, of this department, for their cooperation in this study.

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STUDIES OF THE EFFECT OF INTRAVENOUS ADMINISTRATION OF LIVER EXTRACT IN PATIENTS WITH SICKLE-CELL ANEMIA: AN UNUSUAL RESPONSE*

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A STUDY was undertaken to follow in detail the results of liver extract administered intravenously to patients with sickle-cell anemia.

MATERIAL AND METHODS

Four patients with sickle-cell anemia were studied. The blood was examined for ten to fifty days to establish the range of spontaneous variations. Twenty cubic centimeters of liver extract were administered intravenously, and daily counts were made of both white and red blood cells and reticulocytes for fifteen to forty days. In Case 4 (Fig. 1) additional counts were made at twenty minutes, three hours, five hours, and twelve hours following the administration of liver extract.

Case 1.—T. P., 3 years old, was admitted with pains in the feet and ankles and with mild rhinopharyngitis. X-rays showed marked general cardiac enlargement and osteoporosis of the skull and long bones. Kahn and Wassermann tests were negative. Cholesterol was 110.3 mg. per 100 c.c. of blood, erythrocytes were 2,000,000 per c.mm. with 30 per cent reticulocytes, or 600,000 per c.mm., leucocytes were 10,000 per c.mm. with differential per cent as follows: lymphocytes 35.5, polymorphonuclear cells 54.5, mononuclear cells 6.0, eosinophiles 2.5, basophiles 0.5, and unidentified cells 1.0. Nucleated red blood cells and numerous sickle-cells were present. The blood count was not influenced by the liver extract.

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CASE 2.—W. M. J. was admitted with a leg ulcer of three years' duration and primary syphilis. Electrocardiogram showed slight myocardiac damage. Kahn test of blood was positive. Cholesterol was 139.13 mg., sugar 100 mg., nonprotein nitrogen 28.5 mg. per 100 c.c. of blood. Erythrocytes were 1,900,000 per c.mm. with 22 per cent reticulocytes, or 418,000. Leucocytes were 25,000; differential per cent: lymphocytes 16.0, neutrophiles 61.0, eosinophiles 15.0, monocytes 8.0. Volume index was 1.10. The patient was receiving weekly injections of neoarsphenamine while under observation. The blood and clinical course were not influenced by the administration of liver extract.

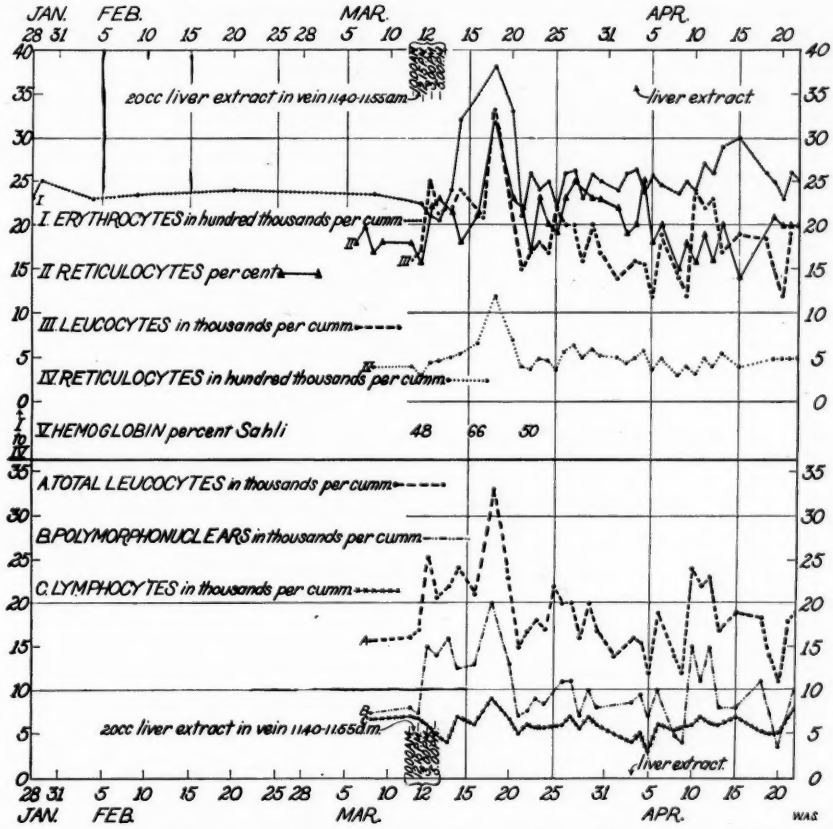

Fig. 1.—Chart showing leucocytes, erythrocytes, and reticulocytes per cubic millimeter and absolute number of the different types of white blood cells in relation to treatment (Case 4, R. R.).

CASE 3.—A. K., 38 years old, was admitted with joint pains and history of similar attacks in the past. He had received antisyphilitic treatment. On February 27 the icteric index was 7.7. Calcium was 9.4 mg., cholesterol 118.5 mg., uric acid 3.7 mg., organic phosphorus 3.6 mg., sugar 91.0 mg., nonprotein nitrogen 27.5 mg. per 100 c.c. of blood. On March 2 bilirubin was 4.16 mg. per 100 c.c. of blood; icteric index was 8.0. Erythrocytes 4,000,000 with reticulocytes 16 per cent, or 640,000 per c.mm. Nucleated erythrocytes were 5,140 per c.mm. Leucocytes were 18,000; differential per cent: lymphocytes 31.0, polymorphonuclear cells 54.0, eosinophiles 3.0, basophiles 2.0, monocytes 10.0. The administration of liver extract did not influence the blood picture.

CASE 4.—R. R., aged 14 years, was admitted with a leg ulcer. He had been admitted 12 years previously during an abdominal crisis. On previous admission (Sydenstricker[1]) he had 1,544,000 erythrocytes with 34 per cent reticulocytes, or 524,000 per c.mm. Leucocytes were 20,000. During present admission his count was erythrocytes 2,300,000 with 18 per cent reticulocytes, or 414,000 per c.mm. Leucocytes were 16,000; differential per cent:

lymphocytes 44.0, neutrophiles 48.0, eosinophiles 3.0, basophiles 1.0, monocytes 4.0 Numerous sickle cells and few nucleated erythrocytes were found. Volume index was 110-118. Three hours after liver extract was administered, leucocytes showed an increase and maintained this rise for nine days. The erythrocytes rose on the third day, and the reticulocytes showed a definite rise on the fifth day. The rise was progressive for all three elements, and on the seventh day they all reached the peak: erythrocytes 3,800,000 with 32 per cent reticulocytes, or 1,200,000 per c.mm. and leucocytes 33,000. On the ninth day the blood count returned to the level noted before treatment. The rise of leucocytes was due entirely to the increase of mature polymorphonuclear cells. The proportion of sickle cells was practically unaltered. The hemoglobin rose, but not in proportion to the rise of the erythrocytes. On the day of administration hemoglobin (Sahli) was 48.0 per cent with 2,300,000 red blood cells; on the fifth day hemoglobin was 66 per cent with 3,555,000 erythrocytes. It returned to the previous level simultaneously with the erythrocytes. Twenty-three days later the same dose of liver was repeated, and on the eighth day a moderate rise of polymorphonuclear cells was observed up to 24,000 per c.mm.; this rise lasted three days. On the eleventh day the erythrocytes rose for two days, but there was no rise in reticulocytes (Fig. 1).

DISCUSSION

Sydenstricker[2] pointed out that the treatment of sickle-cell anemia is most unsatisfactory. Diggs[3] published charts illustrating the negative results in the treatment of sickle-cell anemia by liver extracts.

Following intravenous injection of liver extract, Meyer, Middleton, and Theulis[4] found depression of leucocytes within twenty minutes in normal persons and in patients with blood dyscrasias; this depression was followed by a leucocytosis which reached the maximum three hours later. Miller and Rhodes,[5] in an anemic woman with splenomegaly, found increase of leucocytes in twenty minutes, as well as an increase in the other formed elements of blood, with return to normal in twenty-four hours; the rise of blood count was simultaneous with contraction of the spleen.

The case of R. R. seems to be unique in its response to liver extract. The rise of erythrocytes before the increase in reticulocytes is different from that observed in pernicious anemia. The volume index was slightly higher than normal, but this is frequent in cases of sickle-cell anemia when the reticulocytes are high. The spleen was not palpable and was thought to be greatly diminished, as in sickle-cell anemia. No abnormal blood pressure changes were noted on the day of administration or later. The independent appearance of the rise of leucocytes and erythrocytes, and the duration of this rise, exclude the possibility of its being the result of any kind of redistribution. I feel compelled to consider it the result of action on the hematopoietic system, although the previous administration of liver by mouth and of liver extract intramuscularly was without effect.

SUMMARY

1. The results of the intravenous administration of liver extract to 4 patients with sickle-cell anemia are reported, with 3 patients showing no response and one patient showing unusual response of the hematopoietic system.

2. This response consists in the increase of polymorphonuclear leucocytes, erythrocytes, and reticulocytes on the first, third, and fifth days, respectively, with simultaneous return to previous level on the tenth day.

3. This is considered as the result of action of the extract on the hematopoietic tissue.

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STUDIES IN BLOOD PRESERVATION*

Fate of Cellular Elements and Prothrombin in Citrated Blood

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IN A previous article[2] we reported the changes observed in blood preserved with heparin. The results presented in this study are typical of a large number of observations on citrated blood.

METHOD

Five cubic centimeters of freely flowing venous blood were collected in each of thirty-five sterile flat-bottomed tubes containing 0.5 c.c. of 3.5 per cent sodium citrate solution as the anticoagulant. The blood was kept in a refrigerator at a temperature of from 4° to 6° C. throughout the period of the experiment.

Each day one tube was taken from the refrigerator and the following determinations were made: red blood cell count, hemoglobin, white blood cell count, differential white blood cell count, platelet count, and fragility test.

The plasma clotting time was done on two samples of 50 c.c. of blood by the method of Quick,[7, 8] who postulates that the rate of coagulation is a function of the concentration of prothrombin, and the production of thrombin in oxalated plasma is proportional to the concentration of prothrombin if an excess of thromboplastin is present and an optimal amount of calcium is added. The plasma was recalcified with calcium chloride at a constant temperature of 40° C. in the presence of human brain tissue emulsion to supply the thromboplastin substance, the end point being recorded by the shift on a photoelectric cell galvanometer.

*From the Surgical Pathology Laboratory of the College of Physicians and Surgeons, Columbia University, New York.

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Received for publication, June 1, 1940.

RESULTS

Donor, W. T. S.

COMPARATIVE VALUES OF BLOOD IN HEPARIN AND IN SODIUM CITRATE

	HEPARIN	SODIUM CITRATE 0.35 PER CENT	
Hematocrit (3)	48.5	42.3	per cent cells
Plasma specific gravity (3)	1.0249	1.0238	
Plasma proteins (3)	6.12	5.75	Gm. per cent
Whole blood potassium (10)	192.0	183.0	mg. per cent
Plasma potassium (10)	17.5	17.2	mg. per cent
Cell potassium (calculated) (10)	377.0	409.0	mg. per cent

VALUES ON INITIAL SAMPLE CORRECTED FOR DILUTION IN CITRATE

Red blood cell count	5,100,000.0	
Hemoglobin	15.6	Gm. per cent
White blood cell count	7,700.0	
Differential white blood cell count		
Polymorphonuclear leucocytes	61.0	per cent
Lymphocytes	29.0	per cent
Monocytes	7.0	per cent
Eosinophilic leucocytes	2.5	per cent
Basophilic leucocytes	0.5	per cent
Platelets	206,800.0	

The results of the counts and hemoglobin determinations are graphically represented in Figs. 1, 2, and 3.

Fragility of Erythrocytes.—The end points were poor throughout these studies. An actual curve of fragility could not be constructed on a daily basis. As late as the fifteenth day cells could be suspended in 0.45 per cent sodium chloride without complete hemolysis. Even on the thirtieth day cells carefully handled did not lake completely in 0.52 per cent sodium chloride.

Spontaneous hemolysis was first noted on the seventeenth day. Slight shaking in physiologic saline after the tenth day caused hemolysis. The cells on the tenth day are slightly less resistant than those on the first, and those stored for thirty days are definitely more fragile than those stored for ten days.

Prothrombin.—In the first series plasma clotting times were run for a period of fifty-two consecutive hours at one-hour (toward the end, two-hour) intervals. This was done with the aid of Dr. Kenneth Olsen. There was a rapid rise in clotting times in the first fifteen hours; at the end of this period the prothrombin content had been reduced to ineffectual levels. The curve was similar to that later published by Rhoads and Panzer[9] and seemed to indicate that bloods stored for periods longer than a day would be ineffectual in treating hemorrhage which resulted from a deficiency of prothrombin.

Eight-day-old blood, however, was given a jaundiced patient with a hemorrhagic tendency, and bleeding stopped. This was reported to Dr. Olsen who made up a new extract of rabbit brain and repeated these tests on blood supplied to him from the blood bank. To his surprise this time there was no sudden loss of prothrombin concentration.

Through the courtesy of Dr. Grant Sanger we have been able to obtain the observations tabulated in Table I on separate bloods, each time being very careful to use fresh brain extract.

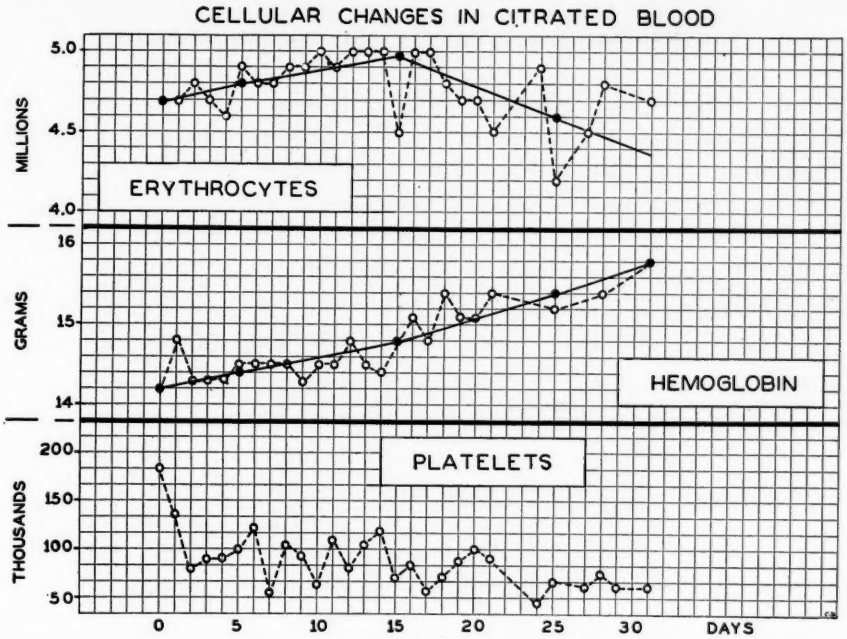

Fig. 1.—Red blood cell counts, corrected for dilution, varied between 5.5 and 4.6, the mean being 5.1 millions. Here there is an actual loss of 1,000,000 to 1,500,000 cells at the end of thirty days. Hemoglobin values varied between 15.6 and 16.3 Gm. per cent. The gradual rise is attributed to evaporation. Platelets fell from 206,800 to 87,800 in forty-eight hours, then remained constant for about fifteen days, after which time counts were difficult.

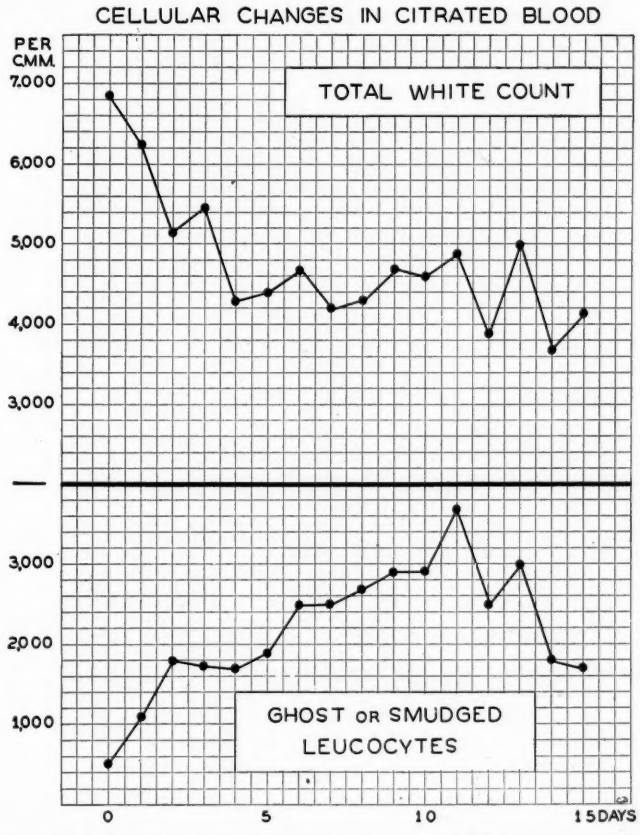

Fig. 2.—The total white blood cell count as observed in the chamber fell 27 per cent in the first five days. Careful differentiation of cells in the chamber and observation on a slide showed that 75 per cent of the total had no nuclei or were so fragile that they broke and left only a smudge by the twelfth day.

DISCUSSION

Red Blood Cells.—The maintenance of total erythrocyte counts at an approximately constant level in heparinized blood[2] and the moderate destruction of erythrocytes in citrated blood after the fifteenth day of storage suggest that cell destruction plays, at most, only a small part in the steady increase of the potassium content of the plasma,[11] and insures the recipient of receiving a large percentage of functioning cells after periods of storage of at least a month.

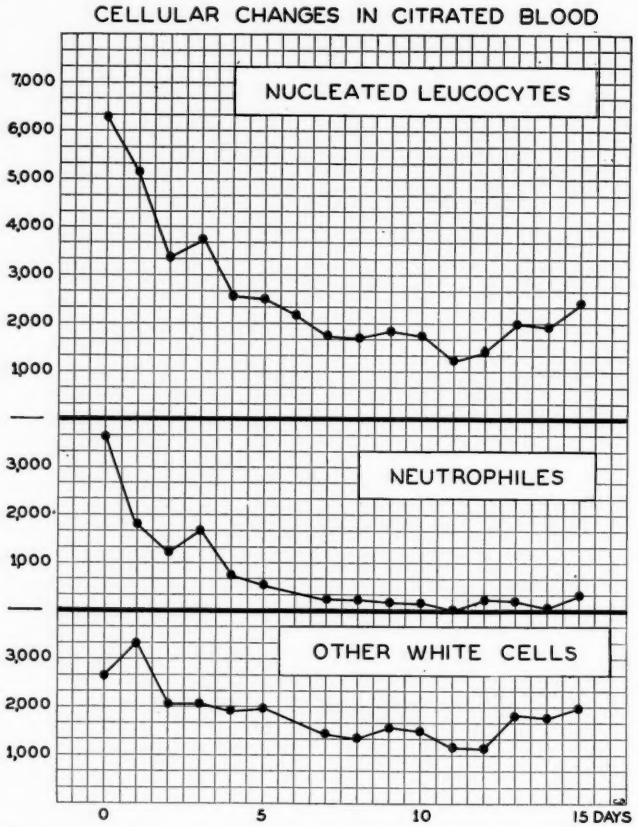

Fig. 3.—The nucleated leucocytes, presumably the only ones capable of function, decreased nearly 50 per cent in the first three days. The polymorphonuclear leucocytes diminished 50 per cent in twenty-four hours, accounting almost completely for the drop in total count. By the sixth day it was difficult to be sure that any remained. Eosinophiles were well preserved. Lymphocytes remained almost constant. Monocytes were difficult to differentiate.

Hemoglobin.—The hemoglobin content of the whole blood remains constant, though at the end of thirty days 20 per cent of it may be found in the plasma.

White Blood Cells.—The polymorphonuclear leucocytes may show swelling, hazy cytoplasm, and poorly staining nuclear granules as early as twenty-four hours after storage. Disintegration is extremely rapid and may be responsible for the steep rise of the potassium curve in the first week;[11] likewise it may play a part in decreasing bactericidal properties.[4, 5]

The lymphocytes are more resistant. At the end of thirty days they are easy to recognize when seen.

The eosinophiles are most resistant, the eosinophilic granules remaining particularly bright even when the nuclear material has faded or broken up.

Platelets.—Platelets disintegrate more rapidly in heparinized blood[2] than in citrated blood. In both bloods they reach their low point in about three to five days. Platelets remaining stay fairly fixed until clumping makes counting difficult about the fifteenth day.

TABLE I

PROTHROMBIN CONCENTRATIONS IN PLASMA OF STORED BLOOD

DATE BLOOD STORED	DATE TEST MADE	AGE IN DAYS	PROTHROMBIN CONCENTRATION (PER CENT)
10/27/39	2/21/40	117	47
10/27/39	2/21/40	117	38
11/20/39	2/21/40	94	41
12/15/39	2/21/40	69	49
1/31/40	2/21/40	21	52
2/28/40	3/12/40	13	100
3/ 1/40	3/12/40	11	100
2/16/40	2/26/40	10	68
2/23/40	3/ 1/40	8	84
2/23/40	3/ 1/40	8	84
2/26/40	3/ 5/40	8	60
3/ 4/40	3/12/40	8	100
3/ 4/40	3/12/40	8	80
3/ 4/40	3/12/40	8	100
2/19/40	2/26/40	7	100
2/19/40	2/26/40	7	76
2/23/40	2/29/40	6	100
2/23/40	2/29/40	6	100
2/16/40	2/21/40	5	74

Prothrombin.—The results using the Quick method of determining the prothrombin concentration yield values similar to those reported by Lord and Pastore[6] using the Brinkhous, Smith, and Warner method.[1] This suggests that the use of brain extract, which has been kept too long, may account for the discrepancy between reported findings. Dr. Olsen deserves credit for making this very essential observation.

SUMMARY

1. In citrated blood there is some loss in the number of red blood cells beginning about the fifteenth day and amounting to from 1,000,000 to 1,500,000 cells by the end of the month.

2. The hemoglobin content remains constant in the total sample, although 15 to 25 per cent may diffuse out of the cells into the plasma in one month.

3. The polymorphonuclear leucocytes are diminished to 50 per cent in forty-eight hours and are amorphous masses in fifteen days.

4. The lymphocytes and eosinophiles do not disintegrate so rapidly; the latter are particularly well preserved. The monocytes are difficult to trace.

5. The platelets fall rapidly to a level ranging from 50,000 to 80,000 and remain at this level for about fifteen days, at which time counts become difficult to make.

6. The fragility of red blood cells slowly increases with increasing age; exact curves are difficult to establish.

7. The prothrombin level is maintained above 40 per cent of normal concentration for a period of at least four months. The use of old brain extract

will cause clotting times which are too rapid, thereby giving a false picture of the true degree of efficacy of preserved blood in the therapy of hemorrhagic diseases associated with low prothrombin concentrations.

We are indebted to Miss Hildegard Menzel for aid in the prothrombin determinations and to Mr. Josiah Lasell for many hematologic studies.

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EFFECT OF VITAMIN C ON WORKERS EXPOSED TO LEAD DUST

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THE present study deals with a group of 303 regular employees, 80 per cent of whom have been in their present place of occupation for five years, and the balance for a longer period. These men are engaged in various types of work in the assembly of passenger automobiles.

The lead exposure in this work of assembly arises from the practice of covering body seams with solder (60 per cent lead), and grinding off the excess metal to form a flush surface. There is thus thrown into the air a large quantity of lead dust which is of variable particle size, the majority of particles being too large to be inhaled.

In a large series of air samples it was determined by standard methods that there was a variable concentration along the assembly line of inhalable lead dust (10 microns or less in size). This concentration varied from 3 to 15 mg. per 10 cubic meters of air.

The concentrations of dust and the particle size findings coincide with the report of Humperdinck[1] on similar lead hazards in European automobile assembly plants.

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Provision for ventilation consists of overhead and floor level exhaust ducts; and grinders and metal finishers are furnished with daily inspected respirators. Floors are kept clean by wet sweeping.

Since early in 1935 all men who were even remotely in contact with lead dust have been examined physically, and a basophilic aggregation count was made (McCord)[2] once a month, or oftener if complaints arose.

During the period in 1935 in which a wave of hysteria concerning lead poisoning was at its height, there were 340 definite complaints that ranged from abdominal cramps, nausea, and vomiting, to alleged weakness of the extensor muscles of the wrists and forearms. The majority of these illnesses were patently of psychologic origin, but there were 13 men in this group who were hospitalized for further observation and possible treatment for lead intoxication.

Urinalysis for lead, both before and after deleading treatment, failed to show an abnormal excretion of lead in any one of these 13 men; and the basophilic aggregation count, with the exception of three of the group, did not bear out a diagnosis of lead intoxication.

The three men who had a high basophilic aggregation were variously found to have a severe pyorrhea alveolaris and chronic cryptic tonsillitis, chronic appendicitis, and peptic ulcer. When treatment appropriate to these conditions was instituted, the blood picture returned to normal, and the lead poisoning disappeared.

At this time a routine form of medication for those exposed to lead dust was initiated. This treatment consisted of the administration once a week—Monday— of 1 ounce of a saturated solution of magnesium sulfate, to which was added 15 grains of citric acid and sufficient tincture of cudbear to color the mixture; this was given to the men when they obtained their tools, thus insuring that practically all the men would receive medication before starting work. The results of this treatment, judged by the basophilic aggregation count and lack of serious complaints, were good, but were met with strenuous objection from the men.

Beginning in June, 1939, the foregoing medication was discontinued, and in its place each man was given two pieces of gum containing a total of 50 mg. of ascorbic acid. These were distributed about one-half hour after the noon lunch period. The men made no objection to taking the gum, and considered it a treat. Physical examinations and basophilic aggregation counts were continued as previously.

After a short period there was a noticeable decrease in muscular fatigue. This was evident by an increase in efficiency and a feeling of well-being at the end of the work period. The men seemed more cheerful and much less inclined to moroseness.

During the five months from June to December, 1939, the basophilic aggregation count showed a slow but progressive decline. At this time further laboratory studies were undertaken as follows: (a) urinalysis for lead, (b) basophilic aggregation count, and (c) a stipple cell count. These determinations were made each week for seven weeks, covering a period of thirty-five working days of eight hours each.

During this test period production was at its peak, humidity was high, and there was a slow circulation of air due to the low temperature of the outside air.

Urine was collected at different periods of the working day to obtain a fair average of lead excretion. A total of 1,996 analyses for lead were made, or an average of 6.5 analyses per man.

The results of the lead urinalysis are shown in Table I.

TABLE I

NO. OF MEN	PER CENT	AVERAGE OF URINARY LEAD EXCRETION PER LITER (γ)
99	32.6	39.5
96	31.6	61.5
48	15.8	86.2
24	7.9	110.4
14	4.6	134.5
10	3.3	160.9
5	1.6	189.6
3	0.9	219.3
1	0.3	265.0
2	0.6	405.0
1	0.3	541.0

Those below 100 γ per liter, 243 men, or 80.2 per cent, averaged 62.4 γ per liter.
Those above 100 γ per liter, 60 men, or 19.8 per cent, averaged 157 γ per liter.
The average over-all excretion of 303 men equaled 77 γ per liter.

In this series was one man who excreted an average of 541 γ per liter. Further questioning revealed that he had had acute lead intoxication several years previously. He had not worked with lead for eight years, and was at least two city blocks away from any lead; therefore, his exposure has been nil. Under the influence of vitamin C, we are evidently releasing the fixed lead from his bones and he is excreting it without showing any clinical symptoms of lead intoxication.

The workers who showed an average of more than 100 γ per liter of urine—60 men—were studied further. The urine and blood counts were continued at fortnightly intervals. At the end of April, 1940, the lead excreted in the urine has shown no decline, and the basophilic aggregation and stipple cell counts have remained within the normal range.

DISCUSSION

From the foregoing it is apparent that this study confirms the contention of Holmes[3] that vitamin C (ascorbic acid) has a detoxifying action on lead in the human body. However, we cannot agree that the urinary lead excretion is decreased by the administration of vitamin C—since our findings show a continued high level of excretion over an observed period of five months in those workers continuously exposed to a high concentration of lead dust.

It is difficult to reconcile our findings with those of Shiels,[4] and Blumberg and Scott[5]; i.e., that there are definite levels of concentrations for lead, both in urine and blood, above which levels, one finds lead poisoning. Judged by the criteria of blood cell counts, physical condition, and lack of symptoms usually associated with chronic lead absorption, the subjects of this study may be said to show no evidence of lead intoxication whatever. Fairhall[6] has stated that a high urinary excretion of lead is not of itself evidence of lead intoxication, but rather of lead absorption only.

We have found no evidence that the storage of lead is promoted by vitamin C. Rather the reverse is true, as shown by the workman with the highest level of excretion in the group. As stated, this man has been previously exposed and has been treated for lead intoxication.

Further investigation in the deleading properties of vitamin C are being undertaken.

It seems possible that the effect of lead on the human economy may be only indirectly toxic, the absorption of lead producing an avitaminosis C. This is the condition of vague symptoms known as chronic lead poisoning, but is in reality chronic subclinical scurvy.

CONCLUSIONS

1. The routine administration of 50 mg. of ascorbic acid daily appears to protect workers exposed to lead dust against the usual effects of chronic lead absorption.

2. The rate of urinary excretion of lead in workers continually exposed to lead dust is not decreased by the routine daily administration of 50 mg. of ascorbic acid.

I wish to express my appreciation to Dr. Milton H. Kronenburg, Chief of Division, and K. M. Morse, Engineer, of the Illinois Industrial Hygiene Bureau, for the air-borne dust determinations; and to Doctors E. K. Nielsen and H. A. Cranston for the determinations of urinary lead.

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A COMPARISON OF THE HEMOGLOBIN RESPONSE TO VARYING DOSAGES OF IRON*

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ALTHOUGH the efficacy of simple iron salts in the treatment of iron deficiency hypochromic anemias has been adequately demonstrated, there is still a question as to the relative effectiveness of various sized dosages. The usually recommended doses are large and the present consensus is that a massive dose of iron is the advisable method of therapy. Balance studies have shown, however, that sufficient iron is retained from the administration of a smaller amount of the drug, so that adequate quantities are available for hemoglobin formation as well as for replenishment of the depleted reserves.[1, 2] From those data it would seem that doses smaller than those usually recommended should produce a satisfactory increase in hemoglobin. The danger of large amounts of iron has been suggested by Elvehjem[3] and others,[4] and although we were unable to demonstrate that iron significantly affected the metabolism of calcium or phosphorus in the adult,[5] the large doses may not be without danger. It was also shown in balance studies that large amounts of iron were retained by the body over long periods of time,[1] and although only one instance of a severe reaction to orally administered iron has been recorded,[6] it does not seem wise to overburden the body with an excess such as must occur with the commonly employed doses when administered over a long period.

It has been demonstrated that large doses of iron produce a more rapid response in hemoglobin formation than do smaller amounts[7-10] and that ferrous salts are more effective than ferric salts when compared on a basis of their metallic iron content.[11, 12] Moore[13] has shown that the serum iron absorption curves are higher following the ingestion of ferrous than of ferric salts. In order to compare the effectiveness of varying sized doses of iron and of various iron salts, we have administered these preparations to groups of patients with mild but comparable degrees of anemia under identical conditions.

From balance studies with iron and ammonium citrates the amount of iron excreted, retained, and utilized from a given dose was known,[1, 2] so that this drug was selected for the first of the clinical trials. All subjects selected were healthy young adults, with no illness except the mild anemia which was detected on routine examination, and none was studied who had had a recent infection, hemorrhage, or other disability which might affect the blood hemoglobin. Hemoglobin determinations were done by the Newcomer method,[14] hematocrits were

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determined by the Van Allen method,[15] and erythrocyte counts were done on instruments certified by the United States Bureau of Standards. In the accompanying charts only the hemoglobin changes are recorded, although hematocrit readings and erythrocyte counts were done at the time of each hemoglobin determination.

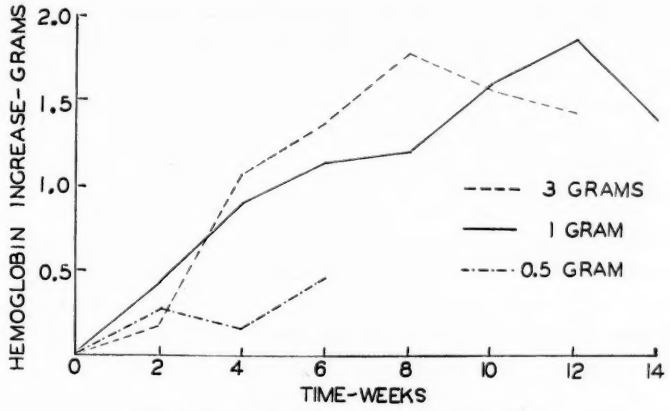

Chart 1.—Iron and ammonium citrates.

To one group of 18 persons was given 0.5 Gm. (7½ grains) of iron and ammonium citrates per day. The average hemoglobin level for this group was 9.73 Gm. per 100 c.c. The average hemoglobin gain for the entire group in a period of six weeks is shown in Chart 1, and it will be noted that although there is an increase in hemoglobin, the gain is too slow to be considered a satisfactory response. During a subsequent six-week period 1.5 mg. of copper sulfate were added to the same dose of iron and the hemoglobin gain was no more rapid than without the additional copper.[16] This amount of iron and ammonium citrates yields approximately 85 mg. of elemental iron per day, but from the results recorded here it is obvious that this amount of iron in the form of iron and ammonium citrates is inadequate to produce a satisfactory clinical response.

To a similar group of 15 persons, whose initial hemoglobin averaged 11.53 Gm., was given 1 Gm. (15 grains) of iron and ammonium citrates per day. This yields approximately 170 mg. of elemental iron per day, and the balance studies had shown that approximately 30 per cent of this, or 53 mg., was retained, and that 9.3 per cent, or 16 mg., was used immediately in hemoglobin formation, so that an abundance of metallic iron was being retained in the body.[2] With the administration of this amount of the drug there was a hemoglobin increase of such degree that the average gain at the end of twelve weeks was 1.82 Gm. per 100 c.c. of blood (Chart 1). Following this, as has been pointed out,[17] there was a gradual reduction in the hemoglobin in spite of continued iron administration. It is well recognized that the rate of hemoglobin regeneration will depend on the degree of anemia as well as on the amount of iron administered. The anemia in this group of persons was mild and the gain of nearly 2 Gm. in the twelve-week period seems very satisfactory, although it is small in comparison with the results obtained in severe anemia.

A third group of 21 persons, with an average hemoglobin of 10.95 Gm., was given 3 Gm. (45 grains) of iron and ammonium citrates per day. This yields approximately 500 mg. of metallic iron per day; it has previously been shown that with this dosage 32.6 per cent of the iron was retained, but only 2 per cent of the amount administered was utilized immediately in hemoglobin formation in a group of 10 patients with an average hemoglobin level of 5.95 Gm.[1] This means that an average of 167.5 mg. of iron was being retained per day, 10.3 mg. was utilized in hemoglobin formation, and that 157.2 mg. was retained in addition to that used immediately in hemoglobin. With this dose of iron and ammonium citrates the average gain for the 21 patients was 1.78 Gm. at the peak of the response, a figure which is very similar to the response obtained with 1 Gm. of the same drug per day. The difference in the two groups lies in the fact that with 3 Gm. per day the peak of the hemoglobin production occurred in the eighth week instead of the twelfth week. The response was more rapid but no greater than with a smaller amount of iron (Chart 1). Following the peak in the hemoglobin response, there was a gradual regression during the following four weeks of observation in spite of continued iron administration.

Similar studies were carried out with small amounts of other simple iron salts for comparison with those obtained with iron and ammonium citrates. Reduced iron, 1 Gm. per day, was given to a group of 7 persons with an average hemoglobin of 11.36 Gm.; it produced a slightly greater increase in hemoglobin than did 1 Gm. of iron and ammonium citrates with the peak of the response in the tenth rather than in the twelfth week. Reduced iron is an insoluble preparation but one which is much higher in its metallic iron content than any other iron salt. As compared with 1 Gm. of iron and ammonium citrate, which contains approximately 170 mg. of metallic iron, 1 Gm. of reduced iron contains nearly 980 mg. of metallic iron so that the greater amount of iron compensates for its insolubility in producing the results shown in Chart 2.

Iron and sodium citrates, 1 Gm. per day, was administered to a group of 13 persons with an average hemoglobin level of 10.72 Gm. The results are shown in Chart 3. The response was somewhat less than with a similar dose of iron and ammonium citrates or reduced iron, but not significantly different.

Both clinical and experimental evidence points to the fact that ferrous salts are more effective than ferric salts when compared on the basis of their metallic iron content. This is substantiated by our results in which the administration of 0.36 Gm. (6 grains) of ferrous sulfate per day given to 11 persons with an average hemoglobin level of 10.58 Gm. produced a hemoglobin response comparable with those obtained with much larger doses of other iron salts (Chart 4). This amount of ferrous sulfate contains approximately 80 mg. of metallic iron as compared with 170 mg. in the smallest effective dose of iron and ammonium citrates and 980 mg. in reduced iron. It is comparable with the metallic iron content in 0.5 Gm. of iron and ammonium citrates which was shown to be ineffective.

It is interesting to note the similarity of the hemoglobin curve in every instance. As was pointed out in a previous communication, the hemoglobin increased to a peak; this was followed by a decline even though the same amount of iron was administered continuously. These persons were not fol-

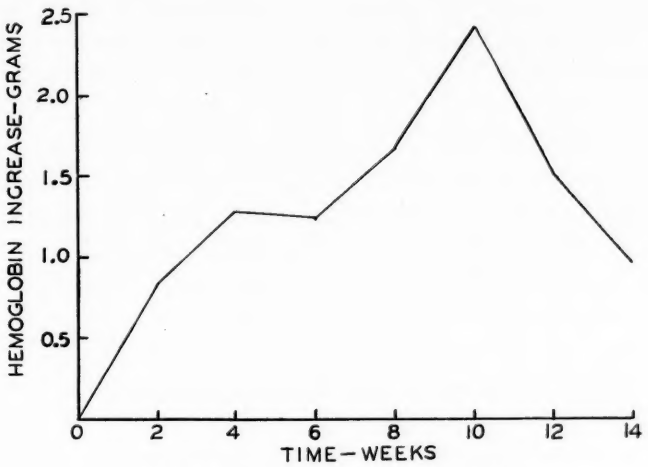

Chart 2.—Reduced iron, 1 Gm. daily.

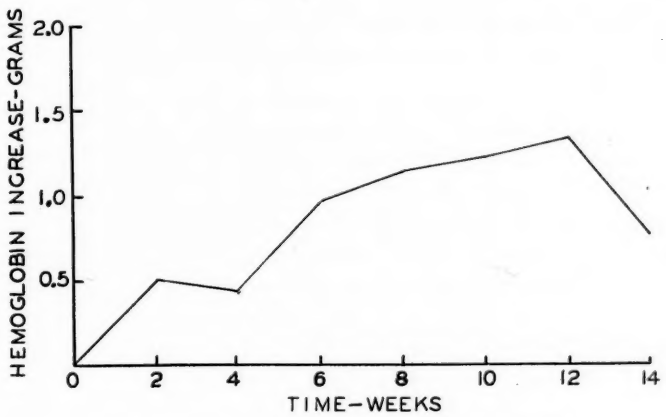

Chart 3.—Iron and sodium citrates, 1 Gm. daily.

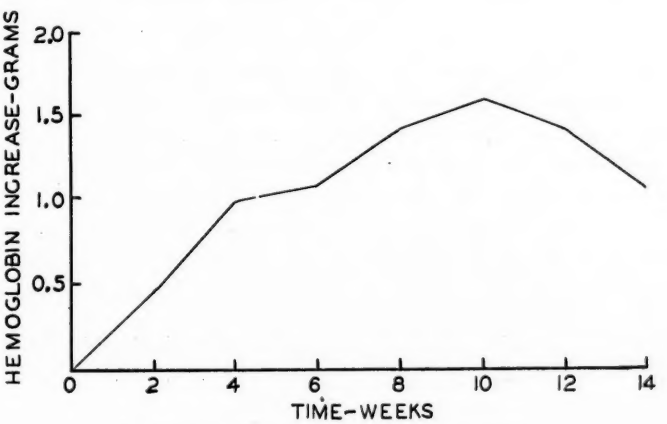

Chart 4.—Ferrous sulfate, 0.36 Gm. daily.

lowed for as long a period as was true in the preceding study, but the hemoglobin curve had essentially the same contour for the shorter period and supports the idea of a stimulating or catalytic effect of iron[10, 18] on hemoglobin formation.

It is useless to speculate on the minimal effective dose of an iron salt because this will vary so greatly in individual patients. Even the commonly employed doses need to be increased in certain cases to procure an adequate response. Hence the results reported here are not intended to be an argument for a wholesale reduction in the amount of iron administered per day to these patients. The renaissance in iron therapy has been due in part to the administration of larger amounts of this mineral, and these larger dosages are advisable in cases of severe anemia and when it is impossible to follow the patient by frequent hemoglobin determinations. It has been shown that 80 per cent of the patients respond satisfactorily to smaller doses[12] so that in the case of mild degrees of anemia or as a maintenance dose in idiopathic hypochromic anemia smaller amounts should be satisfactory for adequate therapy. The results are presented as a comparison of the effectiveness of varying amounts of iron in different iron salts in comparable cases.

SUMMARY AND CONCLUSION

When varying doses of iron and ammonium citrates were employed in the treatment of mild anemias, it was found that 0.5 Gm. per day was too small to be of value. The response to 1 Gm. of the drug per day was satisfactory, although the hemoglobin increase was less rapid than with 3 Gm. of the same preparation per day. Reduced iron, 1 Gm. per day, and iron and sodium citrates, 1 Gm. per day, gave practically the same response as did 1 Gm. of iron and ammonium citrates. Ferrous sulfate, 0.36 Gm. (6 grains) per day, was as effective as 1 Gm. of the other drugs, although the metallic iron content is much lower.

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BLOOD FINDINGS IN LATE PREGNANCY*

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BLOOD findings on pregnant women may not logically be compared with those on normal nonpregnant women. Frequently the differences found between normal and complicated pregnancies are extremely important; as, for example, in toxemia, anemia, or following sulfanilamide therapy. For obstetric work, therefore, standards or ranges based on findings on normal pregnant women should be used.

In Table I we present such data for the carbon dioxide and oxygen contents of the blood, the oxygen capacity, the red blood cell count and volume, and the hemoglobin. The table includes the maximum and minimum findings and the averages from a series of 30 dispensary patients, whose histories and physical examinations indicated that they were normal pregnant women. From these results we have calculated by distribution formulas the range which will include individual findings on all normal pregnant women 95 per cent of the time. For comparison we have included the normal nonpregnant values for these same determinations, compiled from findings published in the literature and accepted by various texts.

The oxygen and carbon dioxide determinations were made, with a few unavoidable exceptions, in duplicate, in a manometric apparatus after the method of Van Slyke and Neill,[1] using oxalated venous blood, drawn free flowing from the arm under oil. Agreement between the duplicates was good, averaging less than 2 per cent difference. The red blood cell counts and red blood cell volumes were determined on the same samples, in the usual manner. Hemoglobin readings were made by the Sahli method, using a standard of 13.8 mg. per 100 c.c. as 100 per cent, and were also calculated from the oxygen capacity by formulas.† Fresh blood was used throughout and all work was completed in from three to five hours from the time the specimen was drawn.

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†Hemoglobin, in grams per 100 c.c. of blood, is found by subtracting the 0.5 volume per cent of physically dissolved oxygen from the oxygen capacity, and multiplying the result by the factor 0.746.

In view of the numerous factors which may affect the oxygen content of venous blood, of which Keys[2] found individual variation the greatest, our values for carbon dioxide and oxygen content are remarkably consistent, showing somewhat less variation than others have reported. Our range and average are a little lower than those Keys found with nonpregnant women, and also less than Eastman's[3] 11 volumes per cent at delivery, or Smith's[4] average of 12.1 volumes per cent for 10 women in labor, before anesthesia or delivery.

TABLE I

	NO. DETERMINATIONS	AVERAGE	RANGE OF ACTUAL DETERMINATIONS	STANDARD DEVIATION	CALCULATED RANGE* (INCLUDES 95 PER CENT OF ALL NORMAL PREGNANT DETERMINATIONS)	NORMAL RANGE FOR NONPREGNANT WOMEN TAKEN FROM THE LITERATURE
CO_2 content in volume per cent	30	47.66	38.66-55.04	3.58	40.50-54.82	45-60
O_2 content in volume per cent	30	6.90	2.80-12.13	2.18	2.55-11.25	11-17.6
O_2 capacity in volume per cent	30	15.25	12.13-19.37	1.79	11.65-18.85	17-20
Erythrocytes per c.mm.	30	3.78	2.94- 4.79	0.53	2.71- 4.84	4.25- 5.00
Red blood cell volume in per cent	29	28.50	22.2 -33.0	2.73	23.04-33.96	35-45
Hemoglobin by O_2 capacity Gm. per 100 c.c.	30	11.00	8.68-14.08	1.35	8.30-13.70	13.0-15.7
Hemoglobin by Sahli Gm. per 100 c.c.	24	11.00	8.42-12.97			13.8

*Two times the standard deviation added to and subtracted from the average gives the range which includes all individual determinations 950 times out of 1,000.

The oxygen capacity determination is more exact, since it is not affected by the factors which tend to vary the content, and is the same for both venous and arterial blood. Here again pregnancy causes a definite lowering from the generally accepted standards. For normal nonpregnant women Peters and Van Slyke[5] give 17 to 21 volumes per cent oxygen capacity; Keys, 17 to 18; and Todd and Sanford,[6] 18 to 24 for both men and women, men being conceded to average higher than women. Our average of 15.25 volumes per cent agrees well with Eastman's 15.4 at delivery, without anesthesia, and is but slightly lower than Smith's average of 16.8 for women in labor.

Oxygen capacity determinations are particularly practical following sulfanilamide therapy. This is illustrated by Table II, where we have presented a brief survey of the laboratory findings on 8 selected patients. Five of these, Nos. 1, 2, 3, 6, and 8, show a serious fall in oxygen capacity to a level below the lower limits, 11.65 volume per cent, of the normal pregnant range—3 by more than 2 volume per cent. In Case 2, even as low a concentration of sulfanilamide as 2.7 mg. per 100 c.c. of blood was sufficient to depress the oxygen capacity. Cases 4 and 7 show declines that were checked by blood transfusions and partial withdrawal of the drug. This decline in oxygen capacity only occasionally follows the use of the drug, but it is always a possibility that should never be

neglected. In the cases where it does occur, the oxygen-carrying power of the cells is apparently blocked, so that an oxygen tent is powerless to relieve the situation. This conclusion is supported by the fact that as the oxygen capacity falls different hemoglobin methods fail to agree, the colorimetric methods, either Sahli or alkaline hematin, invariably giving higher results. Further, there is no correlation between the blood count and the oxygen capacity. Withdrawal of the drug, supported by transfusions, restores the oxygen capacity and the agreement between the hemoglobin findings.

Another effect, illustrated in Cases 2, 4, 5, and 7, is the depression of the carbon dioxide content of the whole blood. This does not parallel the oxygen capacity fall, nor is it exactly proportional to the concentration of the drug in the blood, but it is relieved by withdrawal of the drug or by blood transfusions. No significance has so far been attached to it.

The per cent of oxygen saturation is varied not only by all the factors which affect the content, but also by those which affect the capacity. As is to be expected, our range is quite wide, and little significance can be attached to individual findings. The tendency to lower averages, however, is interesting. Smith reported 71.1 per cent for women in labor; Eastman, 71 per cent at delivery, with no anesthesia; and Keys, 62.8 per cent for nonpregnant women. We find an average of only 44.9 per cent for late pregnancy. Although the red blood cell count varies quite widely and is of less diagnostic value here than in other circumstances, it is consistently lower than that of nonpregnant normal women, averaging 3.77. The red blood cell volume, however, is quite consistent, averaging 28.5 per cent, as opposed to the nonpregnant 40 per cent. The hemoglobin derived from the oxygen capacity naturally parallels those values, and shows little variation. The Sahli findings likewise are very consistent and agree remarkably with the other hemoglobin values, averaging the same. While the findings on a given individual do not always agree, it is worthy of note that no Sahli readings fall outside the calculated range established by the oxygen capacity method for hemoglobin.

There is less interrelation between these different blood findings than might be anticipated. The oxygen and carbon dioxide contents might seem to be inversely proportional, but upon applying Pearson's correlation formulas we find the relation just fails of significance. Neither is there a direct relation between the red blood cell count and red blood cell volume, probably due to variation in the size of the red blood cells, even when applying Pearson's less exact rank formulas, which take into account only the order of magnitude instead of exact values. The oxygen capacity and red blood cell volume did show a bare significance, but in view of the short series reported, this can only be noted as a tendency which might be confirmed in a longer series.

Apparently neither age nor the number of children affects the blood findings. Seven individuals reported were over 25, two were 40, yet none show any significant variation from the general average. Similarly, 11 of the 30 had 2 or more children, one 5, one 6, and one 9; yet these, too, agree with the general averages.

TABLE II

CASE	DATE	CONC. SULFANILAMIDE IN BLOOD (MG./100 C.C.)	WHOLE BLOOD			RED BLOOD CELLS	WHITE BLOOD CELLS	HEMOGLOBIN		REMARKS
			CO_2	OXYGEN				SAHLI (GM./100 C.C.)	O_2 CAPACITY (GM./100 C.C.)	
			CONTENT (VOL. %)	CONTENT (VOL. %)	CAPACITY (VOL. %)					
1. G. D.	6/21/37			12.99	14.74				10.62	At delivery
	6/26/37					2.76	5,400			
	6/28/37	Started					11,200			Alkaline hematin method
	6/29/37			5.17	12.97	3.40			9.30	10.7 Gm. hemoglobin
	6/30/37						7,900			
	7/ 1/37	Discontinued		6.35	10.37	2.51			7.63	8.7 Gm. hemoglobin
	7/ 2/37									Gave 480 c.c. blood
	7/ 3/37			5.30	16.55	3.43			11.97	
2. D. P.	3/14/38	2.7	48.30	5.07	10.52	2.80	16,000	8.56	7.47	
	3/15/38	2.7	42.21	7.36	11.74		11,600		8.61	
	3/16/38	2.2	39.62	8.02	11.76		16,200		8.62	
3. H. J.	11/14/38					3.39		10.90		
	11/21/38					3.11		8.97		
	11/22/38	10.5	45.48	4.78	9.42		10,950		6.65	Gave 500 c.c. blood
	11/23/38	5.4	43.39	8.54	10.85		10,600		7.72	
	11/24/38	Discontinued								
	11/28/38	0.0	55.42	5.67	13.48	3.14	10,000	9.66	9.68	
4. I. E.	11/30/38	Started				2.94	15,800	10.07		
	12/ 1/38	8.7	43.62	8.03	13.69				9.84	
	12/ 2/38	9.1								
	12/ 3/38	8.2	33.60	9.76	13.05	3.45	15,350	10.49	9.36	
	12/ 4/38									Gave 500 c.c. blood
	12/ 5/38	3.9								
	12/ 6/38	4.6				4.41	15,600	12.97		
	12/ 7/38	10.3	39.37	9.27	15.60				11.26	
	12/ 8/38	11.3								
	12/ 9/38	Discontinued								
	12/13/38	0.0	59.70	4.84	16.77				12.14	

5. C. A.	12/18/39	5.8	36.32	8.89	12.96		14,500	9.66	9.30	
6. S. S.	12/30/39	Started								
	1/ 2/40				9.55	2.80	15,100	7.04	6.75	
	1/ 3/40	Discontinued								Gave 500 c.c. blood
	1/ 4/40					3.31		9.66		
7. E. S.	2/27/39					3.07	22,500	9.25		Delivered 2/20/39
	2/28/39				15.41				11.12	
	3/ 1/39	Started			13.44				9.65	Gave 300 c.c. blood
	3/ 2/39	9.0	29.55	7.87	11.72	3.02	16,200	10.21	8.37	Prontosil-2 days
										2 transfusions
	3/ 3/39					3.37	22,800	9.94		2 transfusions
	3/ 4/39		30.30	11.17	13.55	3.95	30,900	11.32	9.74	Blood every 8 hr.
	3/ 5/39	11.1								
	3/ 6/39	10.2	41.47	12.47	16.78				12.14	Died 3/7/39
8. B. S.	4/15/40	10.6	46.21	5.89	11.34	3.08	17,700	10.07	8.09	Delivered 4/10/40
	4/16/40	10.4			9.18	2.79	22,800	8.88	6.47	Gave 300 c.c. blood
	4/18/40					2.78	53,600	8.28		Gave 650 c.c. blood
	4/19/40	0.0			14.51		33,200		10.45	

SUMMARY

Tables of blood findings, including the calculated ranges for normal cases of late pregnancy, are presented.

The practical value of laboratory work for patients on sulfanilamide or allied drug therapy is illustrated with a few selected cases, showing a serious fall in oxygen capacity unrelated to the red blood cell count.

Lack of correlation is demonstrated between the widely varying red blood cell counts and the more uniform red blood cell volumes, while a tendency toward correlation is indicated between the oxygen capacity and the red blood cell volume.

A rough parallel is shown between the colorimetric Sahli hemoglobin readings and those calculated from the oxygen capacity, and thus between the Sahli readings and the oxygen capacity.

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CORRELATED HEMATOLOGIC, PHYSICAL, AND CHEMICAL STUDIES OF THE BLOOD AND STROMA IN POLYCYTHEMIA AND BEEF EMBRYO*

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INVESTIGATIONS of the blood in hemolytic[1] and pernicious anemias[2] have shown marked alterations in erythrocyte composition, and chemical study of posthemolytic residue, or "stroma," prepared from the blood of dogs with experimental disulphide anemia has proved that abnormalities in the chemical structure of the red blood cell may be induced by anemia.[3] Human polycythemia offers a striking hematologic contrast to the anemias; furthermore, it provides an opportunity to secure sufficient human blood for complete hematologic, physical, and chemical investigations, together with the simultaneous preparation and study of the erythrocyte stroma.

Our present objective has been to make a coordinated and as thorough investigation of the blood in polycythemia as possible. The detailed study of a few

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specific cases has been chosen rather than less exhaustive observations upon a larger number of patients. In order to interrelate the hematologic, physical, and chemical characteristics of the blood with that of the stroma or fixed framework of the erythrocyte, a relatively large quantity of blood, from the standpoint of the human subject, must be obtained, despite the use of microchemical methods of analysis. From 30 to 50 ml. of blood are required for all the hematologic, physical, and chemical measurements. An additional quantity (250 to 500 ml.) of blood must be secured for the stroma preparation.* From 5 to 10 Gm. of erythrocyte posthemolytic residue or stroma are required for lipid and amino acid analyses.

The patients of this study have been under clinical observation and treatment for a number of years, and inasmuch as the withdrawal of blood at periodic intervals was the procedure used to control the polycythemia, it offered the first opportunity to make this type of investigation with human beings.

EXPERIMENTAL

Two established cases of primary polycythemia vera and one of cardiac origin were studied. Subject J. S.† (male, aged 49 years) had been under observation for over ten years. Treatment with phenylhydrazine and x-ray had been used, but not in the three years prior to the time at which these studies were made. Subject P. H.‡ (male, aged 31 years) exhibited a mild polycythemia vera, with a red blood cell count never above 7.5 million per cubic millimeter. Subject A. D.‡ (male, aged 31 years) suffered from a congenital heart deficiency and was reported to have been cyanotic since infancy. All three patients were being treated by periodic venesection during the time these studies were made.

Samples of blood for chemical analysis and stroma preparation were removed by venepuncture. Determinations of red blood cell count, hemoglobin, hematocrit, diameter, specific gravity, and water content were made; from these, corpuscular volume, weight, thickness, and hemoglobin and water content were calculated. The chemical data included mineral analyses (sodium, potassium, chloride, calcium, and phosphorus) of the serum and red blood cells; the lipid distribution (total, free, and esterified cholesterol, total phospholipid, lecithin, cephalin, sphingomyelin, cerebrosides, and neutral fat) of the plasma and erythrocytes; and the amino acid and lipid partition of the stroma prepared from the erythrocytes. The hematologic and chemical methods have been outlined in previous studies from this laboratory,[4-6] as has the procedure developed for the preparation of the posthemolytic residue.[7]

*Preparation of posthemolytic residue or stroma involves separation of the fixed framework of the erythrocyte from the hemoglobin. The erythrocytes are separated by centrifugation and washed four times with 4 to 5 volumes of physiologic saline. The washed erythrocytes are then hemolyzed in 20 volumes of sodium citrate buffer (pH 5.0 to 5.5). The material is then run through a Sharples steam centrifuge (30 to 40 thousand r.p.m.), and the precipitate is resuspended in a small volume of the buffer. This suspension is ground in a ball mill in a refrigerator for an hour, after which it is again placed in 20 volumes of buffer and run through the Sharples steam centrifuge. This process is repeated four times, after which the buffer wash solutions show little or no hemoglobin content. The material is then dried in a vacuum oven and powdered.[7]

†We wish to thank Dr. E. A. Sharp of Parke, Davis & Co. and the staff of the anemia clinic of Harper Hospital for their cooperation in making accessible these cases for study, and for their assistance in the collection of the blood samples.

‡We appreciate the courtesy of Dr. Warren B. Cooksey and Dr. Hugo A. Freund in arranging and assisting with the studies on patient P. H.

RESULTS AND DISCUSSION

The hematologic and physical characteristics of the erythrocytes are given in Table I. The average red blood cell of the mild polycythemia vera (P. H.) is of normal size, shape, and weight, in agreement with general literature reports; the volume and weight in the more severe case (J. S.) show some decrease. Both cases demonstrate a decreased quota of hemoglobin per cell. In contrast, the erythrocyte of the cardiac polycythemia (A. D.) exhibits a full quota of hemoglobin and increased volume and weight owing in part to elevated mineral and lipid contents, but chiefly to an augmented water content. Values indicative of the weight of the nonhemoglobin protein of the cell are low for polycythemia vera (1.1 and 2.1 Gm. $\times 10^{-12}$) but normal in the cardiac case (3.3 Gm. $\times 10^{-12}$). Comparable, but slightly lower values, are obtained from the difference between the total nitrogen of the cryoscopically dried cell and that accounted for by the hemoglobin and lipid.

TABLE I

HEMATOLOGIC AND PHYSICAL CHARACTERISTICS OF ERYTHROCYTES IN POLYCYTHEMIA

	NORMAL	POLYCYTHEMIA		
		VERA		CARDIAC
		J. S.	P. H.	A. D.
Hemoglobin (Gm./100 c.c.)	15.4	17.5	15.6	24.3
Red blood cells (millions/c.mm.)	5.4	7.2	6.6	7.6
Hematocrit (per cent)	44	67	60	82
Corpuscular:				
Volume (c.μ)	86	82	90	102
Diameter (μ)	7.7	7.7	7.7	7.6
Thickness (μ)	1.8	1.8	1.9	2.2
Weight ($\times$ 10-12 Gm.)	95	88	97	110
Hemoglobin ($\times$ 10-12 Gm.)	29	21	23	30
Water ($\times$ 10-12 Gm.)	63	63	69	75
Mineral ($\times$ 10-12 Gm.)	0.6	0.6	0.8	0.8
Lipid ($\times$ 10-12 Gm.)	0.4	0.6	0.6	1.1
Nonhemoglobin protein ($\times$ 10-12 Gm.)	1.9	--	1.3	2.1
Undetermined weight ($\times$ 10-12 Gm.)	3.0	2.1	1.1	3.3
Nonhemoglobin protein/lipid ratio	4.9	--	2.3	2.0

The mineral analyses of the serum and erythrocytes are given in Table II. The values for serum water, protein, sodium, potassium, chloride, calcium, and phosphorus are within normal limits. Values for the mineral and lipid composition of the erythrocytes are given in terms of average corpuscular concentration, rather than on the basis of a unit weight of cells, in order to compare the composition of the individual cell in the various conditions.[1, 4] Only in cardiac polycythemia were abnormalities displayed in the erythrocyte content of minerals; increases were found in sodium, chloride, and water. Calculation on the basis of milliequivalents per liter of cell water further demonstrates the elevated chloride and sodium values, and, in addition, a compensatory decrease in potassium.

Data on the lipid distributions of the plasma, cells, and stroma are presented in Table III. A reduced free cholesterol in the plasma of polycythemia vera was a consistent finding. An increased plasma phospholipid with a significantly higher proportion present as cephalin was observed in the patient with cardiac polycythemia. Although Boyd has reported no disturbance in the concentration of plasma lipids in polycythemia,[8] inspection of his data reveals trends similar

to those noted above; i.e., a reduced free cholesterol in polycythemia vera and an elevated phospholipid in secondary polycythemia.

The data demonstrate that the increase in total corpuscular lipid in polycythemia vera is due mainly to an elevated total phospholipid, which is reflected in the cephalin and sphingomyelin fractions. In the patient with cardiac disorder the marked augmentation of corpuscular lipid is accompanied by corresponding increases in free cholesterol and neutral fat as well as total phospholipid. It seems significant, however, that the major increase occurs in the phospholipid and is reflected in all three fractions, cephalin, sphingomyelin, and lecithin.

TABLE II

MINERALS OF SERUM AND ERYTHROCYTES IN POLYCYTHEMIA*

	NORMAL†	POLYCYTHEMIA		
		VERA		CARDIAC
		J. S.	P. H.	A. D.
Plasma protein (Gm./100 c.c.)	7.1	7.2	6.9	8.1
Serum:				
Sodium (meq./liter)	140	140	143	143
Potassium (meq./liter)	4	7	4	7
Chloride (meq./liter)	103	97	101	103
Water (Gm./liter)	944	932	935	936
Erythrocyte:				
Sodium (meq. $\times 10^{-13}$/cell)	15	21	20	44
Potassium (meq. $\times 10^{-13}$/cell)	89	96	133	96
Chloride (meq $\times 10^{-13}$/cell)	45	45	51	70
Water (Gm. $\times 10^{-12}$/cell)	63	63	69	75
Serum:				
Sodium (meq./liter of water)	148	150	153	153
Potassium (meq./liter of water)	4	8	4	7
Chloride (meq./liter of water)	109	104	108	110
Erythrocyte:				
Sodium (meq./liter of water)	25	31	27	54
Potassium (meq./liter of water)	144	141	179	117
Chloride (meq./liter of water)	72	66	69	86

*Values for calcium, inorganic, and acid-soluble phosphorus, in serum and erythrocytes, were normal.

†Values from previous studies.[2, 4]

The increased phospholipid, particularly the cephalin and sphingomyelin fractions, is in striking contrast to the characteristic changes of the erythrocyte composition in severe anemia;[1, 2] it is similar, however, to the chemical alterations induced in the red blood cells by liver therapy in pernicious anemia during the period of active reticulocyte response.[2, 9] There seems to be no evidence, therefore, of an accumulation of old cells exhibiting fatty degeneration; rather, the mass presence of young cell forms and active bone marrow stimulation.* These findings are in agreement with hematologic observations of the presence of immature red blood cell forms in the circulation[10, 11] and the histologic evidence of increased erythroblastic activity of the bone marrow in polycythemia.[12, 13]

The augmented lipid content of the erythrocyte may be indicative of a greater amount of fixed lipid structure, inasmuch as experimental data have

*An increased span for beginning and complete hemolysis by hypotonic sodium chloride solutions gave evidence of the presence of cells of varied ages, particularly immature forms.

The periodic venesection may have had some effect upon bone marrow activity and the type of erythrocytes released into circulation.

TABLE III

DISTRIBUTION OF LIPIDS IN BLOOD AND STROMA IN POLYCYTHEMIA

	PLASMA (MG./100 C.C.)				ERYTHROCYTE (MG. × 10^{-12}/CELL)				STROMA (PER CENT DRY WEIGHT)					
	NORMAL	POLYCYTHEMIA			NORMAL	POLYCYTHEMIA			NORMAL	POLYCYTHEMIA			BEEF	
	HUMAN*	VERA		CARDIAC	HUMAN	VERA		CARDIAC	HUMAN	VERA		CARDIAC	NORMAL*	EMBRYO‡
		J. S.	P. H.†	A. D.		J. S.	P. H.†	A. D.		J. S.§	P. H.†	A. D.		
R. B. C. (millions/c.mm.)					5.4	8.9	7.2	7.6						
Total lipid	750	721	420	796	399	631	561	1072	14.1	18.4	20.0	21.5	25.6	19.5
Cholesterol:														
Free	82	48	37	73	92	136	109	199	2.3	4.9	5.2	5.7	6.9	5.1
Esters	254	198	180	322	30	0	39	0	0.5	0.1	0.7	0.5	0.6	0.4
Phospholipid:														
Total	189	245	130	303	233	407	359	618	9.6	10.0	12.0	12.6	16.0	11.9
Lecithin	99	158	50	--	70	74	61	171	2.0	3.6	2.4	3.2	--	2.2
Cephalin	55	66	43	210	173	244	218	359	4.6	3.4	4.5	6.9	7.8	6.3
Sphingomyelin	35	21	37	--	45	89	82	88	3.1	3.0	5.1	2.5	--	3.1
Cerebrosides	15	11	0	27	43	76	35	73	1.7	1.6	2.1	2.5	2.1	1.6
Neutral fat	210	220	80	71	5	13	19	169	0	1.8	0	0.1		0.5

*Values from previous studies.[2, 4, 6, 14]
†Average of 2 samples.
‡Average of 6 samples.
§Average of 4 samples.

shown that practically all of the lipid in the intact erythrocyte is combined with the stroma.[14] The posthemolytic residue prepared from the erythrocytes in polycythemia exhibited an elevation of total lipid, chiefly cholesterol (Table III). An augmented phospholipid content was found in the preparations from P. H. and A. D. and was reflected in the sphingomyelin and cephalin fractions, respectively. The differences between cells and stroma in the phospholipid partition indicate that a portion of that present in the whole cell is not combined with the stroma. These lipid anomalies are diametrically different from those found in the red blood cell stroma of dogs subjected to experimental N-propyl disulfide anemia, in which reductions of total lipid, phospholipid, and cholesterol were observed.[3]

The lipid distribution of the posthemolytic residue of embryonic beef erythrocytes (Fig. 6) has been included (Table III) in view of suggestions that polycythemia vera may be analogous to fetal polycythemia.[13] No similarities between the lipid abnormalities of the embryonic beef and polycythemia vera erythrocytes are evident. In fact, the embryonic preparations, in comparison with stroma from erythrocytes of adult beef, exhibit lower total lipid, free cholesterol, and phospholipid, differences which are suggestive of the anemias, and recall the hematologic similarities of fetal and pernicious anemia erythrocytes.[15]

TABLE IV

AMINO ACID ANALYSES OF ERYTHROCYTE POSTHEMOLYTIC RESIDUE IN POLYCYTHEMIA AND BEEF EMBRYOS

(MILLIMOLS PER 100 GM. OF PROTEIN*)

	NORMAL HUMAN	POLYCYTHEMIA			BEEF	
		VERA		CARDIAC	NORMAL†	EMBRYO§
		J. S.‡	P. H.	A. D.		
Arginine	27.6	26.6 ± 0.6	27.4	19.5	29.3	29.0 ± 2.7
Histidine	13.5	11.7 ± 1.7	8.7	13.5	12.3	11.5 ± 1.3
Lysine	26.0	24.8 ± 2.0	26.8	21.0	24.0	31.0 ± 2.4
Cysteine	7.5	9.9 ± 0.8	8.0	8.8	7.4	9.8 ± 0.8
Tyrosine	14.9	15.3 ± 0.6	15.6	--	16.0	16.9 ± 2.0
Tryptophane	5.9	5.8 ± 0.3	5.6	--	5.9	5.7 ± 0.5
Total nitrogen (per cent)	13.0	13.1 ± 0.4	12.6	13.7	13.8	13.7 ± 0.2

*Analyses on alcohol ether extracted preparation.
†Values from previous studies.[6]
‡Average of 4 preparations with average deviation from the mean.
§Average of 5 preparations with average deviation from the mean.

The protein fraction of the posthemolytic residue has been analyzed for six amino acids: arginine, histidine, lysine, cysteine, tyrosine, and tryptophane. Since these amino acids do not have uniform molecular weights or nitrogen contents, the analyses are expressed in terms of millimols per 100 Gm. of protein in Table IV. In polycythemia vera less histidine was found than is present in normal stroma; in the severe case (J. S.) the decreased histidine was accompanied by an increase in cysteine content. The stroma protein from the cardiac polycythemia erythrocytes contained more cysteine but less arginine and lysine than normal.

If 35,000 is taken arbitrarily as the molecular weight of the stroma protein, then a deviation from the normal of 2.86 millimols of an amino acid per 100 Gm. of protein represents a variation of 1 Gm. mol of that amino acid per 35,000 Gm. of protein, and may be considered a significant difference. By this criterion only

cysteine differs significantly (one-half to 1 mol) in the preparations of J. S., while histidine is lowered by 1 to 2 Gm. mols per 35,000 Gm. protein in the stroma of P. H. In the cardiac case, both arginine and lysine are diminished significantly (2 mols).

TABLE V

SIGNIFICANCE OF DIFFERENCES* IN AMINO ACID ANALYSIS

(MILLIMOLS PER 100 GM. OF PROTEIN)

	POLYCYTHEMIA			EMBRYONIC BEEF
	VERA		CARDIAC	
	J. S.	P. H.	A. D.	
Arginine	−0.9	−0.1	−8.0	−0.3
Histidine	−1.8	−4.8	0	−0.7
Lysine	−1.2	+0.8	−5.0	+7.0
Cysteine	+2.4	+0.5	+1.3	+3.3
Tyrosine	+0.4	+0.7	–	+0.9
Tryptophane	0	−0.3	–	−0.2

*A variation from the normal of 2.86 millimols per 100 Gm. of protein is equivalent to 1 mol of an amino acid per 35,000 Gm. of protein (the assumed molecular weight), and therefore, a difference of this magnitude or larger may be considered significant.

The amino acid analyses of the embryonic beef erythrocyte residues* exhibit significant differences in comparison with preparations from adult beef erythrocytes, but differences which are not similar to those displayed in polycythemia. Outstanding is the increase of the lysine fraction by 2 mols, and the cysteine by 1 mol.

This evidence of abnormalities in the amino acid composition of stroma in polycythemia is unique, in view of previous studies which have shown that posthemolytic residue preparations from the erythrocytes of 5 mammalian species were practically identical in amino acid composition.[6] Furthermore, no alterations were found in the amino acid composition of red blood cell stroma of dogs with experimental disulfide anemia.[3]

SUMMARY

Correlated hematologic, physical, and chemical investigations of the blood plasma and erythrocytes have been made in two cases of polycythemia vera and one of secondary polycythemia. These studies have been accompanied by detailed lipid and amino acid analyses of the erythrocyte posthemolytic residue or stroma. Analyses on the stroma of embryonic beef erythrocytes are included for comparison.

The data indicate significant alterations not only in the composition of the intact erythrocytes but also in the lipid and amino acid constitution of their so-called fixed structure or stroma. Increased corpuscular total lipid and phospholipid, accompanied by corresponding increases of cephalin and sphingomyelin were the salient characteristics. The posthemolytic residues prepared from the erythrocytes exhibited an elevation of total lipid and cholesterol. The data indicate, however, that not all the augmented phospholipid evident in the cell in polycythemia is bound with the stroma.

*These differences in composition of the erythrocyte residue of embryo bloods from that of the normal adult have suggested a study which is now in progress: the pattern of the chemical composition of tissues during growth.

No similarities were evident between the stroma from the erythrocytes of beef embryos and polycythemia in their lipid anomalies.

Significant, but dissimilar, differences were found in the amino acid composition of the erythrocyte stroma protein from polycythemia vera, cardiac polycythemia, and embryonic beef preparations.

The cardiac polycythemia exhibited more marked and extensive changes in the mineral and lipid composition of the erythrocyte as well as the lipid and amino acid make-up of the posthemolytic residue.

These data in polycythemia add further evidence to those in the anemias that the stage of maturation of the erythrocyte may be reflected closely in its lipid composition and that of the posthemolytic residue.

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THE CHEMISTRY OF INFECTIOUS DISEASES*

I. Polarographic Study of Blood Sera in Experimental Pneumonia in Dogs

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Brdicka[1] reported that carcinomatous serum gave a typical polarographic curve, differing from that given by normal serum, by which early cases of malignant growth could be detected. Waldschmidt-Leitz,[2] in a study of over 500 cases, confirmed this observation. A check on the polarographic findings by clinical methods revealed that less than 10 per cent of the polarographic diagnoses were incorrect. Of these the wrong positive diagnoses were always of a deep-seated inflammatory nature, which prompted us to investigate the influence of severe pneumococcal inflammations on the normal polarographic curve of serum.

EXPERIMENTAL

All dogs were infected† by the method of Terrell, Robertson, and Coggeshall[3] by the direct introduction of type I pneumococcus culture‡ into one lobe of the lung after the dog's body temperature had been lowered 3° to 4° F. by the subcutaneous injection into the groin of a saline solution of 6 mg. of morphine sulfate per kilogram of body weight.

After infection periodic samples of 5 to 8 c.c. of blood from either the cephalic or the external jugular veins were taken every three to four hours for the first thirty hours after infection, later twice daily, and then once until the dog had recovered or died. The sera were obtained in the usual way from the clotted and centrifuged blood.

Plenty of water was available to the animals at all times. A sufficient amount of our usual dog diet (horse meat and Purina Dog Chow) was given once a day. During the first and second days after infection the dogs often did not eat food.

A Heyrovsky self-recording polarograph was used throughout this work. The buffer solution for the serum analyses was similar to the one recommended by Brdicka.[1] It was prepared as follows:

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†We gratefully acknowledge the invaluable help and the unstinted cooperation we received in this study from Dr. W. Harry Feinstone of this laboratory. Dr. Feinstone, while actively studying related phases of pneumococcal infection in dogs, kindly took over the culturing of the pneumococci and the actual infection of the dogs, and followed the progress of the disease systematically with x-ray photographs.

‡Furnished through the courtesy of Dr. O. H. Robertson, of the University of Chicago.

10 c.c. of a cobaltic hexammino-chloride solution (0.2776 Gm. in 100 c.c. H_2O; decomposes within a few hours)
5 c.c. 1 N ammonium chloride
15 c.c. 1 N ammonium hydroxide
70 c.c. water

100 c.c. solution

In the analyses of whole serum, 0.45 c.c. of water was added to 0.3 c.c. of serum, and 0.1 c.c. of this diluted serum solution was electrolyzed in the presence of 10 c.c. of the cobalt buffer. The current-voltage curves were recorded in all cases from –0.9 to –1.7 volts. The sensitivity of the instrument was set at $\frac{1}{200}$, with an accumulator voltage of 4.0 volts. The dropping rate of the mercury electrode was 2.8 seconds per drop into the buffer solution at zero potential.

A variation from this procedure, also recommended by Brdicka[1] and referred to in this paper as "peptone fraction," consisted in the denaturation of 0.4 c.c. of serum with 1 c.c. of 1 N potassium hydroxide for exactly forty-five minutes, then precipitation of the macroproteins with 1 c.c. of 20 per cent sulfosalicylic acid and filtration after ten minutes' standing. One-half cubic centimeter of the filtrate was added to 5 c.c. of the cobalt buffer for electrolysis. The current-voltage curves were recorded as described under whole serum, except that the sensitivity was set at $\frac{1}{100}$.

The solid content of serum was determined by drying exactly 1 c.c. of serum in a weighing flask to constant weight in a vacuum desiccator over phosphorus pentoxide at room temperature.

TABLE I

AVERAGE, MAXIMUM, AND MINIMUM VALUES OF THE DIFFERENCES IN POLAROGRAPHIC WAVE HEIGHT BETWEEN –1.65 AND –1.06 VOLTS OF FIVE DOGS INFECTED WITH TYPE I PNEUMOCOCCUS

Differences between –1.65 and –1.06 volts in centimeters.

DOG NO.	NO. OF DETERMINATIONS	NORMAL DOGS			INFECTED DOGS
		AVERAGE	MAXIMUM	MINIMUM	MINIMUM
I	10	2.19	2.28	1.96	1.89
II	10	2.13	2.29	1.96	1.86
III	16	1.99	2.44	1.89	1.68
VI	18	2.15	2.35	1.97	1.69
VIII	3	2.04	2.14	1.96	1.68

RESULTS AND DISCUSSION

A polarographic wave of the whole serum of Dog VI prior to infection is recorded in Fig. 1, curve I, while Fig. 1, curve II, is the corresponding curve of the serum at the height of infection. It can be seen from the two figures that the minimum at –1.65 volts in the case of the normal serum is higher in relation to the flat part of the curve at –1.06 volts than the corresponding minimum of the infected serum. We selected, as a base line, the value at –1.06 volts, even though it is on a slope, since it represents the maximum of the current-voltage curve of the cobalt-buffer solution in the absence of proteins, and because it is very easily reproducible for a given serum. If this difference is plotted against

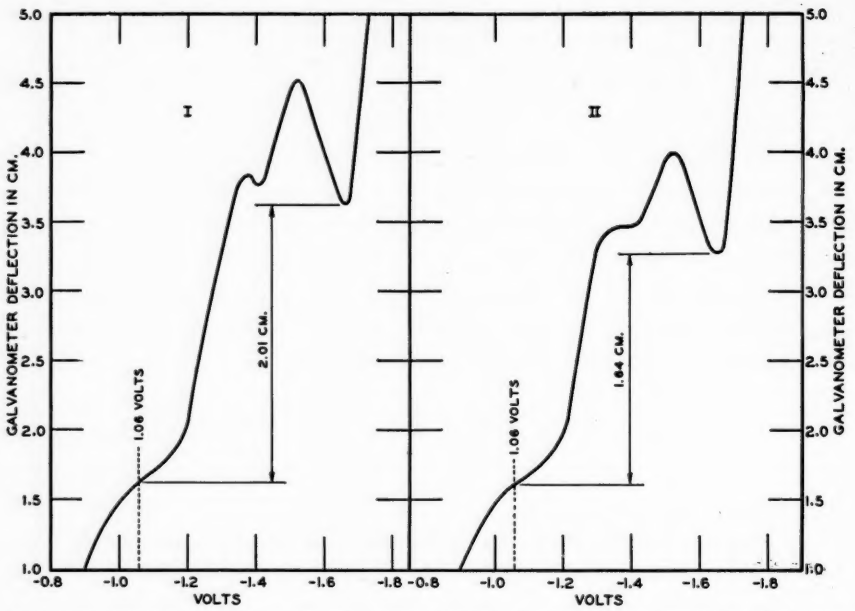

Fig. 1.—Polarograms of whole dog sera before (curve I) and after (curve II) infection with type I pneumococcus.

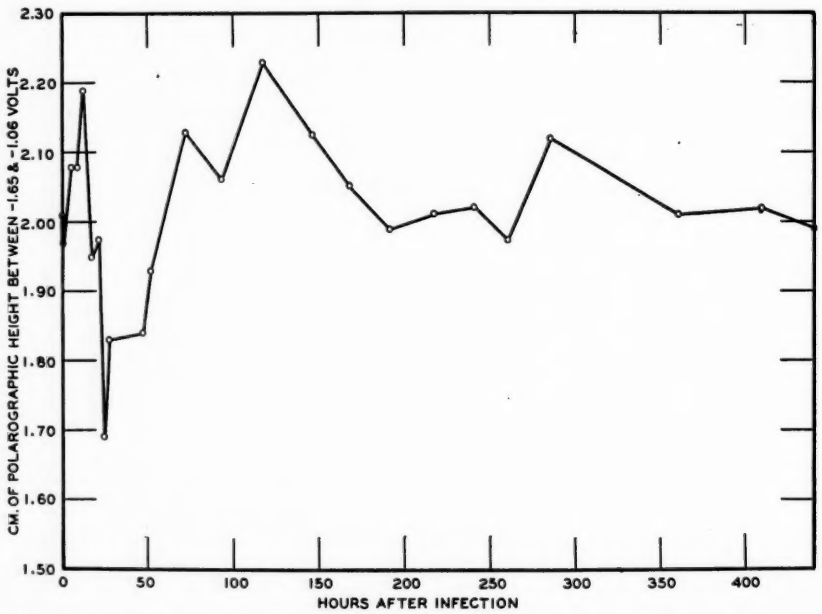

Fig. 2.—Polarographic wave height of whole dog sera after infection with type I pneumococcus.

time, i.e., hours after infection, a curve such as shown in Fig. 2 for Dog VI, is obtained. During the first thirty to fifty hours after infection, a sharp drop in the wave height occurred in all the five infected dogs studied. The return to normal values is then very rapid. However, the decrease is not uniform and loses much of its value due to the fluctuations in the wave height of the normal sera of the same dog, as shown in Table I, which summarizes the distances in

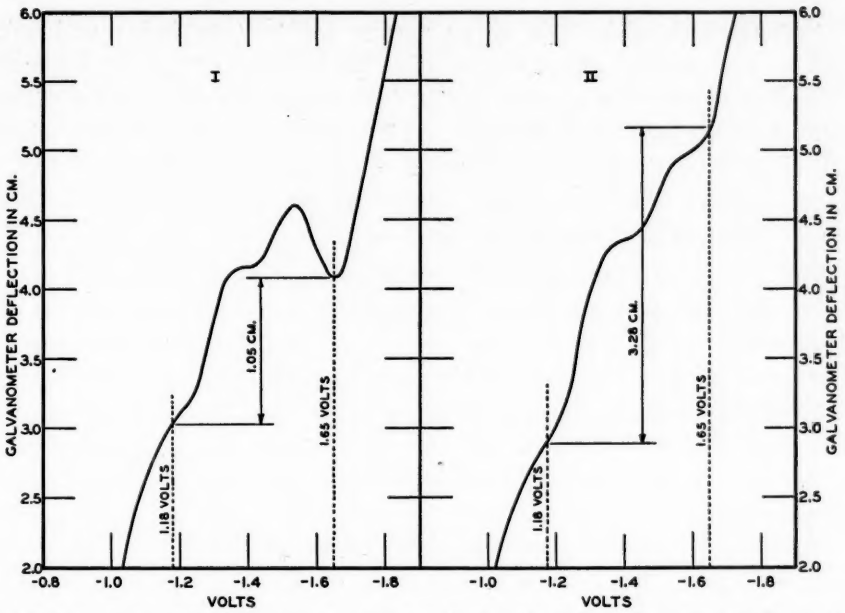

Fig. 3.—Polarograms of the filtrates of potassium hydroxide denatured, sulfosalicylic acid precipitated dog sera before (curve I) and after (curve II) infection with type I pneumococcus.

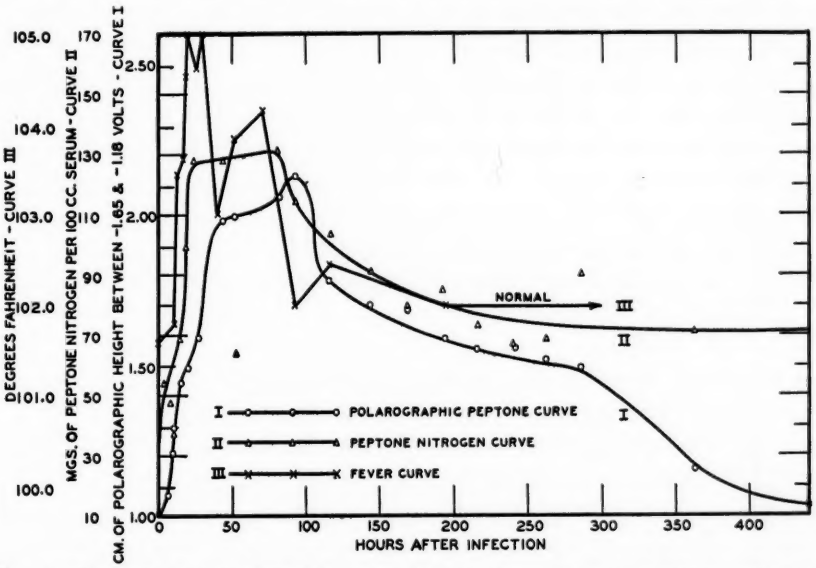
DEGREES FAHRENHEIT - CURVE III
MGS. OF PEPTONE NITROGEN PER 100 CC. SERUM - CURVE II
CM. OF POLAROGRAPHIC HEIGHT BETWEEN -1.65 & -1.18 VOLTS - CURVE I
I POLAROGRAPHIC PEPTONE CURVE
II PEPTONE NITROGEN CURVE
III FEVER CURVE
NORMAL
HOURS AFTER INFECTION

Fig. 4.—Changes in polarographic wave height (curve I) and nitrogen content (curve II) of the peptone fraction of sera (i.e., filtrate from potassium hydroxide denaturation and sulfosalicylic acid precipitation) and body temperatures (curve III) of the same dog with time after infection with type I pneumococcus.

centimeters between –1.65 volts and –1.05 volts of the five infected dogs at the height of the illness, as well as the averages, maximum and minimum values of the corresponding normal blood samples.

The decrease in wave height, nevertheless, is real, reproducible, and undoubtedly represents some change in the serum with infection. Probably it is due to a decrease in proteic sulfhydryl and disulfide groups, i.e., a decrease in cystine (and cysteine) in peptide linkage, for we confirmed Brdicka's observa-

tion[4] that proteins with little or no cystine sulfur (protamine and gelatin) as well as free cystine and cysteine, give no typical protein double wave in the trivalent cobalt buffer, while glutathione produces slight, and cystine-rich proteins a pronounced, double wave. The addition of free cystine either to serum or to crystalline serum albumin, buffered with trivalent cobalt, also does not increase the wave height from that given by the same amount of serum or albumin without the cystine.

While the polarograms of whole sera decrease in wave height with infection, probably indicating concentration changes, the current-voltage curves of the peptone fraction not only increase in wave height, but also undergo a pronounced alteration in the slope of the curves. This is clearly demonstrated in Fig. 3, curves I and II, which represent enlarged reproductions of the polarograms of normal and infected peptone fractions, respectively, of blood sera of Dog VI. It should be observed that the most pronounced changes in appearance of the curves occur at –1.65 volts. With an increase in infection the pronounced minimum of the double wave of normal serum gradually flattens out until, during severe infections, the curve at –1.40 volts becomes almost a straight line sloping upward. As the infection recedes, the height of the wave decreases until, upon complete recovery, the typical curve of a normal serum is obtained. If the difference in the peptone wave height between –1.65 volts and –1.18 volts is plotted against time after infection, a curve such as shown in Fig. 4, curve I (Dog VI), is obtained. The reason for choosing –1.18 volts as a base line, even though it falls on a slope, is that the value is constant in all normal dogs and changes very little during the entire period of infection. The peptone-fraction graphs of seven infected dogs show the same general tendency as the one represented in Fig. 4, curve I, for Dog VI, the only differences being that the absolute values of the peaks in the wave height, as well as the exact time after infection at which the peaks occur, vary with each dog. After recovery from the illness all seven dog curves return to their normal values, which lie between 0.85 and 1.25 cm. wave height difference (1.65-1.18 volts), while the peaks reached by the seven infected dogs, together with the respective times after infection at which they occurred (in parenthesis), are: 2.61 cm. (144 hours), 2.20 cm. (127 hours), 2.14 cm. (92 hours), 2.13 cm. (93 hours), 2.11 cm. (144 hours), 2.00 cm. (46 hours), and 1.61 cm. (102 hours). The greatly increased wave height of the peak in the first-mentioned animal is very interesting, since the dog died during the night after this particular blood sample had been taken. Profound alterations in the serum of this dog must have occurred, and speculations as to the relationship between the changes in the serum, which are measurable by the polarograph, and to the cause of death, are very attractive and worthy of a continued study of this problem.

The observed decrease in the galvanometer deflection when whole serum is studied amounts to 10 to 15 per cent of the normal value. This change can undoubtedly be attributed to changes in the solid content of the serum, for a dilution of the serum of from 8 to 13 per cent is found in the case of two of the dogs studied. At least part of this decrease in solid content must be

attributed to nitrogenous constituents, for the total nitrogen values drop from 1,000-1,100 mg. per cent to 800-950 mg. per cent.

The nitrogen values of the peptone fraction, on the other hand, increase during infection by about 100 per cent and parallel closely, in time and percentage, the changes in the polarographic wave height. This can be seen in Fig. 4, curves I and II, where for better comparison the polarographic peptone wave height, as well as the milligrams of peptone nitrogen of Dog VI, are plotted against time after infection. Curve III in Fig. 4 represents degrees of fever during the infection. It is felt that while the nitrogen values agree fairly well with the changes in polarographic wave height, the same corollary with fever does not hold nearly so well. At least fever is not the direct cause of the observed polarographic changes, since the onset, rise, and fall in the fever curves of all seven dogs occur much sooner and more rapidly than the polarographic changes.

SUMMARY

Polarographic studies of normal and of type I pneumococcus-infected dog sera are presented.

The wave height of the current-voltage curves of whole sera decrease during infection and return to normal with recovery. It is felt that these changes may be associated with corresponding shifts in the proteic-cystine content of the sera.

The polarograms of the peptone fraction of dog sera, i.e., that fraction which, after alkali denaturation, is not precipitated by sulfosalicylic acid, increase in wave height as well as change their shape during infection. Recovery is associated with a return to normal values. With the increase in wave height during illness, a corresponding increase in nitrogen values occurs, while the fever curves do not directly parallel the polarographic changes.

We are greatly indebted to Mr. O. E. Sundberg, of the Calco Chemical Division of the American Cyanamid Co., for his cooperation in determining all the serum nitrogen values reported in this paper by the micro-Kjeldahl method.

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STUDIES IN DYSTROPHIA MYOTONICA*

VI. Results of Glucose Tolerance Tests

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THE hypothesis has been advanced that the muscular changes in dystrophia myotonica are of endocrine origin; certainly many of the later symptoms of the disease suggest endocrine dysfunction. Defects of carbohydrate metabolism[6] and storage[8-10] have also been suggested as the cause of the weakness and atrophy of the muscles. A study of the glucose tolerance in these patients might furnish some information about the state of the endocrine system and carbohydrate metabolism; differences in capillary and venous blood glucose during glucose tolerance tests might give some clue to the utilization of glucose by the tissues; a variation in response to different doses of glucose might disclose borderline pathologic change. Accordingly, observations were made on both capillary and venous blood following the ingestion by each patient of two different doses of glucose.

Previous reports on the subject of fasting blood sugars and sugar tolerance tests in the muscular dystrophies and other severe muscular disorders are meager and often conflicting. In cases with *dystrophia myotonica* Schranke and Full[1] reported no glycosuria following 150 Gm. of glucose (1 case); Brock and Kay,[2] a normal fasting glycemia and no glycosuria following 200 Gm. of glucose (1 case); Keschner and Finesilver,[3] a prolonged hyperglycemia following breakfast (1 case); d'Antona,[4] a fasting hypoglycemia (2 cases); a fasting hyperglycemia (1 case); a normal, a low prolonged, and a high prolonged glucose tolerance test (1 case each); Morgulis and Spencer,[5] consistent zigzag tolerance curves with glycosuria in some instances. In cases of *muscular dystrophy*† Janney, Goodheart, and Isaacson[6] found a lasting hypoglycemia (9 cases); McCrudden and Sargent,[7] fasting hypoglycemia (3 cases, progressive type); Hughes and Shrivastava,[8] hyperglycemia following 50 Gm. of glucose (2 cases, Erb's type); Braestrup,[9] normal fasting glucose (3 cases); prolonged hyperglycemia and normal capillary-venous blood sugar differences following 1 Gm. of glucose per kilogram body weight (2 cases); normal glycemia and no capillary-venous differences (1 case). In cases with *myasthenia gravis* McCrudden and Sargent[7] reported a slight fasting hypoglycemia (2 cases); Williams and Dyke,[10] a normal tolerance test following 50 Gm. of glucose but a prolonged

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†In early, and even in more recent reports, dystrophia myotonica has not always been distinguished from other muscular dystrophies. From the descriptions given by Janney, Goodheart, and Isaacson, it appears quite likely that at least two of their patients may have had dystrophia myotonica. Likewise, the patient described by Brock and Kay undoubtedly had dystrophia myotonica rather than myotonia congenita.

hyperglycemia following 100 Gm. (3 cases); Adams, Power, and Boothby,[11] normal glycemia (5 cases). In one case with *progressive muscular atrophy* Braestrup[9] found no capillary-venous differences during the glucose tolerance test.

METHOD

The 7 patients used in this study have been described in a previous article of this series.[12] The controls, in the same age groups as those with dystrophia myotonica, were 5 clinic patients and one of us (M. R.), carefully examined and found normal. None of the persons used in these studies was undergoing any kind of treatment at the time of the test or for a week previously.

The experiments were performed in the morning on persons who had received no food since the preceding evening. Blood was withdrawn from the brachial vein through a small needle into a syringe and immediately transferred from the tip of the syringe to two Folin[13] blood pipettes calibrated "to contain"; the blood was discharged into two tubes of acid cadmium solution, and in the pipettes were thoroughly rinsed by drawing the solution up into them several times. Duplicate samples were then taken from a finger tip* directly into two Folin blood pipettes and discharged into the acid cadmium solution. An average of 3.4 minutes (range two to seven minutes) elapsed between the completion of the venous sampling and the completion of the capillary sampling. After a sample of urine had been collected, the patient received the measured amount of glucose dissolved in 250 c.c. of water flavored with lemon juice. Blood and urine samples were again taken at one-half, one, two, and three hours after the ingestion of glucose. All patients spent the time during the test in reading, resting, and walking about the halls. The test was performed twice within two weeks on each person, once with 50 Gm. of glucose and once with 100 Gm., though not necessarily in this order. Blood sugar values were determined by the Miller and Van Slyke[15] method. All solutions were repeatedly rechecked or standardized as required; all volumetric apparatus was carefully calibrated at the beginning of the work. Duplicate analyses were performed in all the experiments and gave an average difference of 2.6 mg. per cent, with a range of 0 to 9 mg. per cent.

RESULTS

In all cases the fasting level of the blood was normal and no differences were evident between the controls and the patients in this respect. No glycosuria occurred in any of the tests. To conserve space, the individual data on the controls, which gave normal glucose tolerance curves in all respects at both levels of glucose, have merely been summarized in Table I. Since the rise in blood sugar is greater in the capillary blood than in the venous blood, two tolerance curves, one for the venous and one for the capillary blood, were obtained for each test, but the interpretation of the results of these tests is similar.

Results With 50 Gm. of Glucose.—The results for patients with 50 Gm. of glucose are very similar to those for the controls. The fasting values rise sharply

*Foster[14] demonstrated that in studies on blood glucose, cutaneous blood could be substituted for pure arterial blood.

TABLE I

GLUCOSE TOLERANCE TESTS ON PATIENTS WITH DYSTROPHIA MYOTONICA

CASE	SEX	AGE	FASTING		30		60		120		180	
			CAP.	VEN.	CAP.	VEN.	CAP.	VEN.	CAP.	VEN.	CAP.	VEN.
J. B.	♂	60	79.2*	76.5	112.1	99.8	136.0	113.8	134.3	113.3	109.5	99.8
			81.1	*86.2*	*115.2*	*110.0*	*133.8*	*114.5*	*118.3*	*97.7*	*110.5*	*82.3*
N. P.	♂	54	89.0	96.5	160.8	123.3	126.6	94.8	101.0	92.8	80.5	73.0
			89.5	*83.5*	*164.6*	*119.5*	*159.2*	*139.6*	*161.9*	*127.5*	*105.2*	*85.5*
J. M.	♀	46	92.5	81.6	130.5	108.0	100.5	72.5	94.5	81.5	71.1	72.0
			82.5	*84.1*	*143.2*	*127.6*	*107.8*	*99.6*	*102.1*	*94.5*	*109.8*	*98.0*
F. B.	♂	44	87.2	86.9	132.7	116.5	156.3	138.3	114.3	105.3	76.6	74.3
			85.7	*84.3*	*150.4*	*134.0*	*148.3*	*135.3*	*133.6*	*134.5*	*124.1*	*125.8*
R. B.	♂	28	77.4	81.2	129.7	111.0	101.4	80.6	88.5	82.1	67.3	66.1
			77.4	*77.0*	*130.6*	*95.7*	*103.1*	*76.9*	*95.7*	*60.6*	*87.3*	*63.5*
O. M.	♂	43	82.9	83.1	124.8	112.0	80.4	52.5	86.3	73.1	70.5	68.0
			85.5	*85.1*	*129.3*	*106.6*	*78.7*	*53.0*	*91.6*	*67.1*	*73.3*	*68.9*
N. M.	♀	58	88.0	84.7	145.0	115.2	123.8	95.8	108.7	103.8	78.8	77.3
			96.2	*93.8*	*135.0*	*102.5*	*142.0*	*91.0*	*138.0*	*108.5*	*116.0*	*104.0*
Patients, 50 Gm. avg.			85.2	84.4	133.7	112.3	117.9	92.6	103.9	93.1	79.2	75.8
Patients, 100 Gm. avg.			*85.4*	*84.9*	*138.3*	*113.7*	*124.7*	*101.4*	*120.2*	*98.6*	*103.7*	*89.7*
Controls, 50 Gm. avg.			87.1	87.3	148.6	126.4	140.4	115.9	91.7	82.7	77.0	76.0
Controls, 100 Gm. avg.			*86.0*	*85.5*	*148.1*	*122.7*	*119.7*	*95.3*	*113.1*	*94.9*	*78.7*	*73.5*

*Figures in roman type represent values obtained with 50 Gm. of glucose; those in italics with 100 Gm. of glucose. Blood values as milligrams per cent at minutes noted.

from normal to a peak, which is usually reached at thirty minutes but may be delayed until sixty minutes; the values then decline at the end of the third hour to below the fasting level, with one exception (J. B.). The peaks of the curves in both venous and capillary blood average about 15 mg. per cent less than the corresponding controls, but the variations from individual to individual in both patients and controls are so great that these differences scarcely can be considered significant. The peaks for the venous curves appear somewhat low, with a range of 108.0 to 138.3 and an average of 117.4 mg. per cent. However, since this represents an average increase of approximately 39 per cent over the fasting level, and since the smallest percentage increase is 26.7, these curves can be considered normal.

Comparison of the Results Obtained Following 100 Gm. of Glucose With Those Obtained Following 50 Gm.—MacLean and de Wesselow,[16] and Williams and Dyke,[10] found that in normal persons increasing the dose of sugar over about 25 to 50 Gm. gave no increase in the peak of the resulting hyperglycemia but tended to prolong the period during which the blood sugar was raised. Gray[17] noted that the variations in glucose tolerance curves were as great from individual to individual as from different sized doses. Strouse[18] reported that blood sugar values obtained in normal persons by giving 100 or 150 Gm. of glucose could be considerably increased in the same individuals by 200 Gm. of glucose, and Williams and Dyke[10] found a difference in the glucose tolerance curves obtained with 50 and 100 Gm. of glucose in 3 patients with myasthenia gravis,

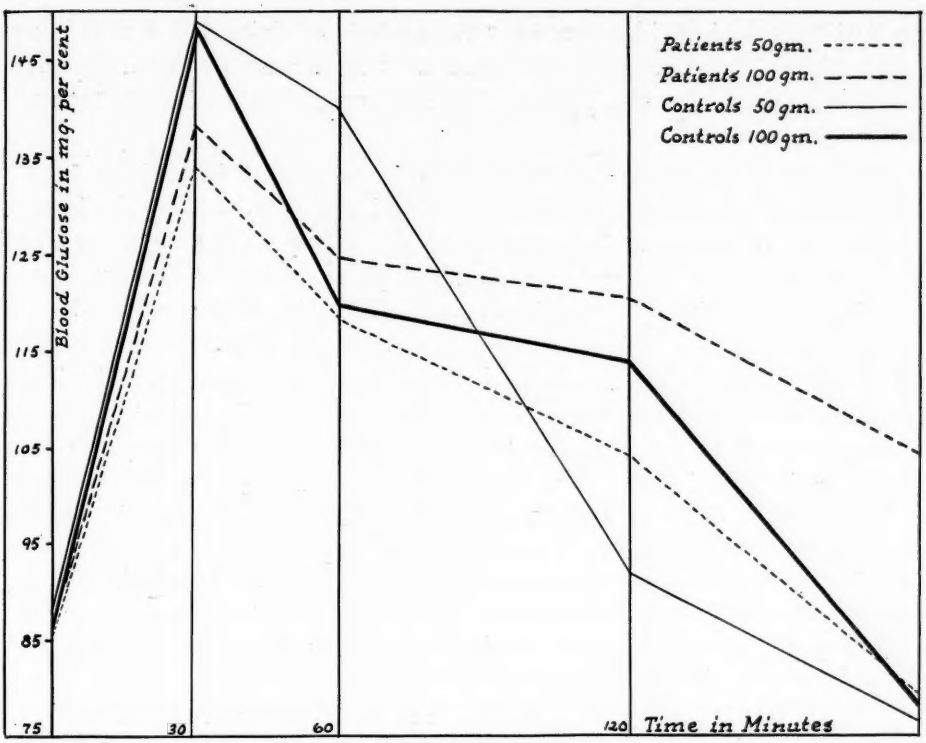

Chart 1.—Glucose tolerance curves on capillary blood.

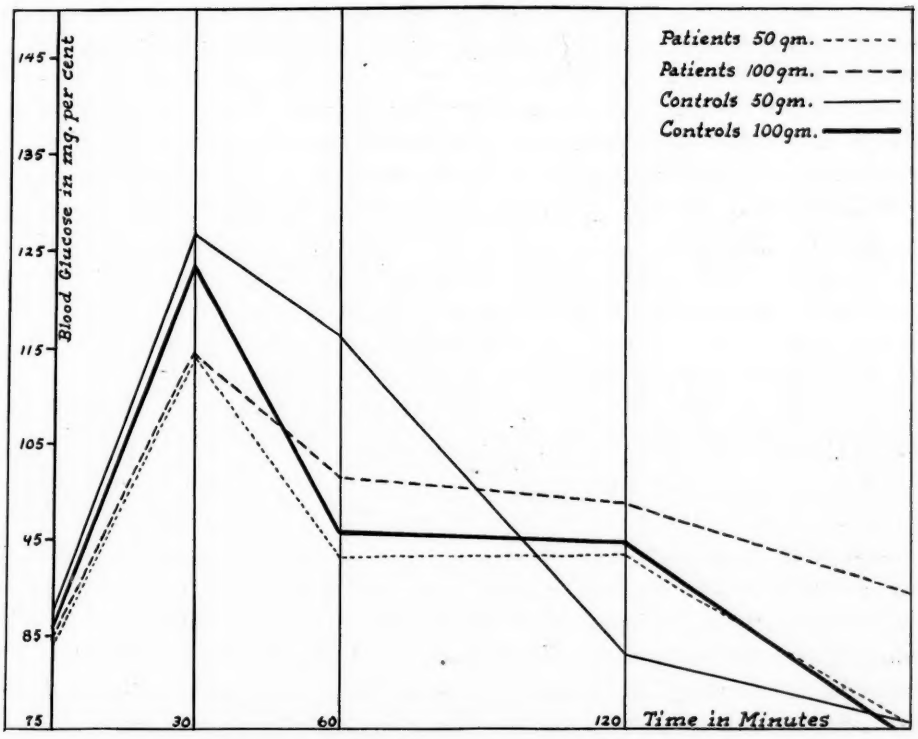

Chart 2.—Glucose tolerance curves on venous blood.

Although the individual curves of the controls receiving 100 Gm. of glucose show variations,* the composite curves are similar to those produced by 50 Gm. and give little evidence of a more prolonged hyperglycemia with 100 than with 50 Gm. of glucose.

The composite curves following 100 Gm. of glucose in patients tend to remain somewhat elevated during the second and third hours as compared with 50 Gm. (Table I and Charts 1 and 2). Thus, in the capillary bloods the final values with 100 Gm. average about 25 mg. per cent above the corresponding values with 50 Gm.; in the venous bloods, they average about 14 mg. per cent higher. This slight prolongation of the hyperglycemia with 100 Gm. is also evident when the final blood glucose values are compared with the initial values: at the 50 Gm. level only one patient had a higher final than fasting blood sugar (J. B. on venous and capillary blood); at the 100 Gm. level, 6 patients had higher final than fasting values (N. P., J. M., F. B., and N. M. on venous and capillary blood; J. B. and R. B. on capillary blood only). While the two levels of glucose gave somewhat different results in the patients than in the controls, the number of cases is so small and the variations from individual to individual are so marked that one cannot say whether these different results are merely chance or are indicative of a somewhat prolonged hyperglycemia at the higher glucose level.

Zigzag curves, usually less marked than those described by Morgulis and Spencer[5] in patients with dystrophia myotonica, occurred among both controls and patients. Although somewhat more numerous in the patients than in the controls, their rather frequent occurrence in routine glucose tolerance tests indicates that these curves are of no special significance in dystrophia myotonica.

TABLE II

AVERAGE CAPILLARY-VENOUS BLOOD GLUCOSE DIFFERENCES

SUBJECTS, DOSE	FASTING	30	60	90	120
Controls, 50 Gm.	0.2*	22.2	24.5	9.0	1.0
Controls, 100 Gm.	0.5	25.4	24.4	18.2	5.2
Patients, 50 Gm.	0.8	21.4	25.3	10.8	3.4
Patients, 100 Gm.	−0.5	24.6	23.3	21.6	14.0

*Blood values as milligrams per cent at minutes noted.

Capillary-Venous Differences.—Our data for the capillary-venous blood sugar differences show practically identical results for both patients and controls after the ingestion of 50 Gm. of glucose (Table II). In both cases the differences are zero or very small at the fasting level; they rise to an average maximum of about 25, with a range of 18 to 31.8 mg. per cent during the first hour, as a rule, and then approach zero at the end of the third hour. These figures are almost an average for the arterial-venous differences reported in the literature (Foster;[14, 19] Lundsgaard and Holbøll;[20] Rabinowitch;[21] Friedenson, Rosenbaum, Thalheimer, and Peters;[22] and Cavett and Seljeskog[23]). With 100 Gm. the capillary-venous differences decline to their original level somewhat more slowly, especially in the case of the patients (see Charts 1 and 2); how-

*In considering our data on the two levels of glucose, care must be taken to compare venous values with the corresponding venous values; the same is true of the capillary values; obviously the latter will be much higher throughout the hyperglycemia than the former.

ever, no statistically significant difference is evident between the controls and the patients receiving 100 Gm. of glucose. One control showed the maximum difference at the end of the second hour in both tests; one patient (J. B. at 100 Gm.), the most severely affected clinically, showed a progressively increasing difference through the third hour—a result of special interest, since no capillary-venous difference might have been expected if severe muscular atrophy were a factor in limiting this difference.

SUMMARY

Glucose tolerance tests were performed on 7 patients with dystrophia myotonica and on 6 controls. In each patient the test was performed once with 50 Gm. of glucose and once with 100 Gm., and in each test both capillary and venous blood were examined.

The fasting blood sugars and the glucose tolerance curves were normal in all patients; no glycosuria was evident. Composite glucose tolerance curves showed slight differences between patients and controls: (1) The glucose tolerance curves were slightly flatter in the patients than in the controls. (2) The composite curves for 100 Gm. tended to remain elevated in the patients at the second and third hours, especially in the capillary blood. (3) The capillary-venous differences remained slightly higher in patients than in controls receiving 100 Gm. of glucose. None of these differences was great enough to be statistically significant, and no correlation was found between variations in response to the tests and the severity of the disease.

Since marked dysfunction of the islands of Langerhans, the anterior pituitary gland, or the adrenal cortex is usually reflected in the fasting blood sugar and in the sugar tolerance curve, the results obtained furnish some evidence against a marked involvement of these endocrine glands. The removal of glucose from the capillary blood by the tissues, as indicated by the capillary-venous differences, also appears normal. It may be concluded that the mechanisms controlling the disposal of ingested glucose in dystrophia myotonica are normal.

We wish to express our appreciation to Dr. James J. Waring for pointing out the value of the glucose tolerance test in dystrophia myotonica and to him and Dr. Robert C. Lewis for their many helpful suggestions during the progress of this work and the preparation of this manuscript.

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LABORATORY METHODS

GENERAL

A VESSEL FOR THE MEASUREMENT OF pH AND OXIDATION-REDUCTION POTENTIAL OF BACTERIAL CULTURES*

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Philip L. Varney, Ph.D., St. Louis, Mo.

THE determination of the changes of the pH and the oxidation-reduction potential which a culture of bacteria undergoes during the course of its metabolism is of considerable interest to workers concerned with the problem of the physiology of bacteria. The difficulty of following these changes over more or less lengthy periods of time without the danger of contamination has interested us in designing the culture vessel described below.

The details of construction are evident in Fig. 1, and consist essentially of three electrodes, calomel, glass, and platinum, sealed in a No. 40 standard taper, pyrex inside joint by means of red sealing wax. These electrodes dip into the bacterial culture contained in the vessel constructed from the outside member of a No. 40 standard taper ground joint. By appropriate means the pH may be obtained from the voltage difference between the glass and calomel electrodes. The oxidation-reduction potential may be measured by using the calomel and platinum electrodes; or under special conditions, by the glass and platinum electrodes described by Wynd.[3]

Electrode *1* is a saturated potassium chloride: calomel half cell. The side arm *G* passes through the base of the standard joint, as indicated, and dips into the culture. The contact *H* is made through a small piece of porcelain bacteriologic filter candle sealed into the end of the tube *G*.

Electrode *2* is a double shank glass electrode constructed as described by Varney.[2] The bulb is filled with 0.1 N hydrochloric acid and contains a silver: silver chloride electrode whose external contact is indicated by the numeral 2 in the figure. The silver wire passes through a small glass tube of about 3 mm. outside diameter, which is sealed into the inner tube *B* of the glass electrode by red sealing wax. *C* is the outer shank of the glass electrode unit by means of which a very high degree of electrical insulation is obtained.

Electrode *3* furnishes the platinum surface *D*, whose simple construction requires no comment.

The culture vessel itself contains about 50 c.c. of bacterial suspension. The side arm *O* may be closed with a cotton plug, or a steel ball bearing may be inserted to permit anaerobic conditions and allow for the escape of carbon

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dioxide. It is necessary first to clear the culture vessel of oxygen by passing nitrogen through the apparatus by means of the opening in the plug of stopcock *N*. The degree of anaerobiosis obtained is sufficient for many types of bacteria. Side arm *P* and stopcock *N*, are so constructed as to permit the removal of uncontaminated drops of the culture at intervals without disturbing the electrode arrangement. Various types of side arms may be constructed for the practical manipulation of various types of experiments.

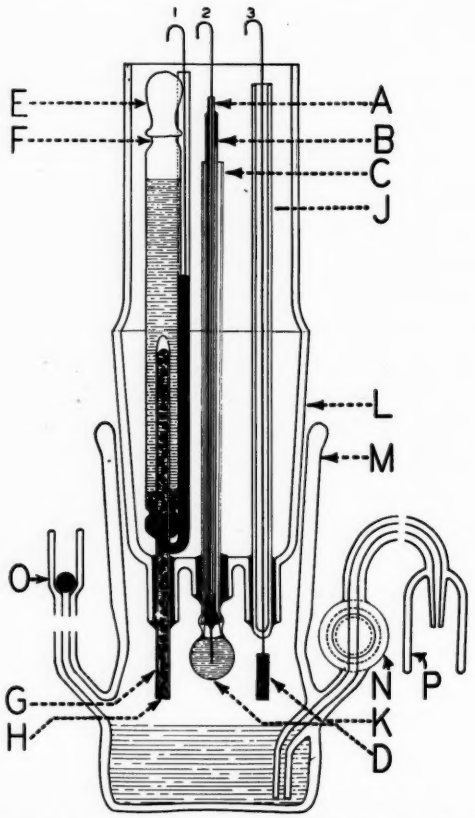

Fig. 1.—Culture vessel and arrangement of the electrodes. *1*, Contact of the calomel half cell; *2*, contact of the glass electrode; *3*, contact of the platinum electrode; *A*, capillary tube filled with red wax and carrying the silver wire into the bulb of the glass electrode; *B*, the inner shank of the glass electrode which seals the outer shank *C* at the base; *D*, bulb of the glass electrode containing 0.1 N hydrochloric acid; *E*, rubber bulb closing the calomel half cell; *F*, the calomel half cell; *G*, side arm of the calomel half cell filled with saturated potassium chloride containing crystals of potassium chloride; *H*, piece of porcelain filter candle sealed into the glass side arm of the calomel half cell; *J*, shank of the platinum electrode; *K*, platinum foil; *L*, inside member of a No. 40 standard taper ground joint; *M*, outside member of the ground joint; *N*, ungreased stopcock; *O*, ball bearing or cotton plug; *P*, cup protecting the enclosed tip from contamination.

The technique of operation is simple but must be carefully done. The vessel *M* and its side arm *O* are plugged with cotton and autoclaved. The structure *L* is sterilized by dipping it into a solution of calcium hypochlorite for a few seconds, washed with sterile water, and placed in sterile water in a duplicate vessel *M* until ready for use. It is advisable to permit the electrodes to stand in the sterile water overnight in an incubator at 37° C. to insure the complete removal of the calcium hypochlorite from the electrically active surfaces. The culture is next added to the vessel, and the electrodes are quickly inserted.

The series of culture vessels is then connected to the proper contacts by hooking the wires *1*, *2*, *3*, shown in Fig. 1 over the spring brass wires shown in Fig. 2. This device is constructed on a bakelite base and the electrical contacts are screwed to the upper ends of six inch polystyrene insulators. (The wiring is arranged as shown in Fig. 3.) The calomel contact *1*, in Fig. 1, hooks over the spring wire *P*, the glass electrode contact *2*, in Fig. 1, hooks over wire G, and the platinum electrode contact *3*, in Fig. 1, connects to the spring wire C.

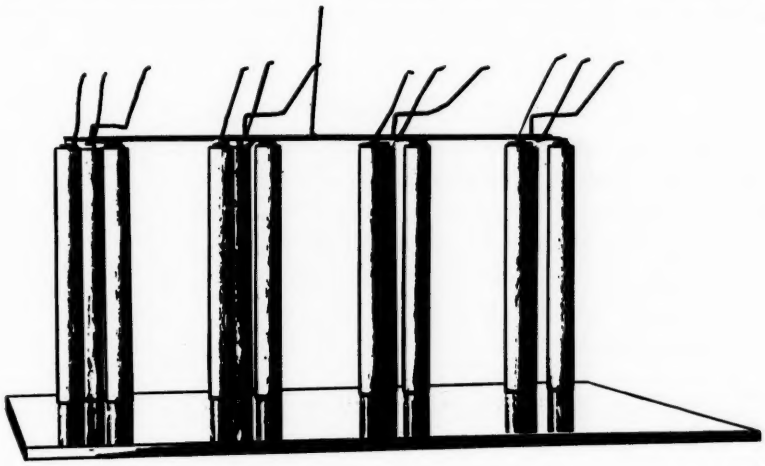

Fig. 2.—Series of electrode contacts within the incubator.

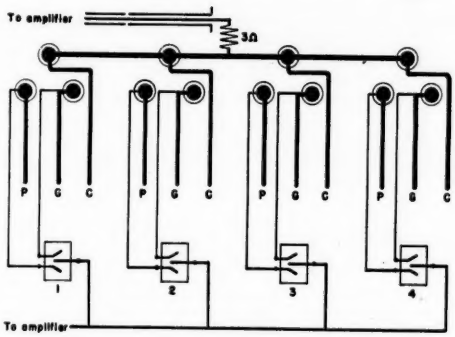

Fig. 3.

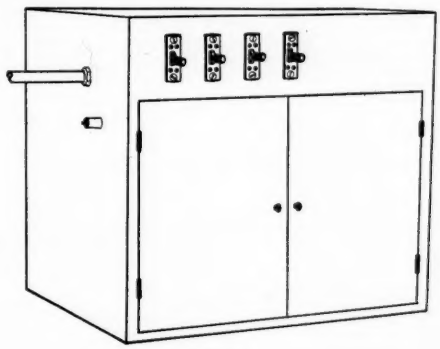

Fig. 4.

Fig. 3.—Wiring diagram of the electrode platform shown in Fig. 2.
Fig. 4.—Incubator chamber, showing the arrangement of the control switches and the leads to the amplifier.

The complete electrode platform rests on porcelain insulators inside the incubator shown in Fig. 4, This incubator chamber is constructed of sheet iron or duralumin. The Federal Switches shown in Fig. 4 are those indicated as *1*, *2*, *3*, *4*, in Fig. 3. The common lead from the four calomel electrodes is attached to the control grid of a vacuum tube amplifier by passing through a pyrex tube, as shown on the end of the incubator. The common lead from the central contact of the four Federal Switches, as shown in Fig. 3, is brought to the proper terminal of the amplifier by means of the porcelain insulator shown on the end of the incubator.

The current from the various pairs of electrodes is amplified by the usual vacuum tube apparatus, and the voltage is read from a potentiometer. The type of amplifier described by Skow and Wynd,[1] and by Wynd and Varney,[4] functions very satisfactorily when used in this manner.

Examination of Figs. 3 and 4 shows that the pH, or the oxidation-reduction potential of any of the four cultures may be obtained conveniently by the proper manipulation of the Federal Switches and by the observation of the corresponding voltage. By means of this apparatus we have successfully followed the changes in pH and the oxidation-reduction potential of bacterial cultures over periods as long as six days.

SUMMARY

1. The pH and the oxidation-reduction potential of a series of bacterial cultures may be observed over comparatively long periods, without disturbing the cultures, by means of a series of vessels within a metal incubator, each containing a glass, platinum, and calomel electrode, properly connected to a vacuum tube amplifier through the wall of the incubator.

2. Observations have been obtained successfully over periods as long as six days without removing the cultures from the incubator.

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A SIMPLIFIED WESTERGREN SEDIMENTATION RATE TECHNIQUE

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THE Westergren sedimentation tube has been modified by various people to facilitate the technique of the test. All of the modifications, however, require extra apparatus, such as stopcocks, racks, or special pipettes. We have been using a technique that in our opinion is simpler, requires no additional parts, except a rubber diaphragm, and is accurate.

TECHNIQUE

The diaphragm used is simply the rubber bulb of a small dropper, such as the one supplied with sample nose drops. The end of the rubber bulb is cut off and the base is placed over the bottom of the Westergren tube. The blood is drawn from the vein in the usual manner, and the proper amount of anticoagulant is then drawn into the same syringe. Then after introducing some air, the blood and anticoagulant are mixed by gentle agitation of the syringe. The

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mixture is then introduced into the Westergren tube by inserting the needle through the rubber diaphragm. By using this technique the level of the blood in the tube can be carefully regulated. On withdrawal of the needle the rubber diaphragm seals itself off. With this procedure the intermediate steps between

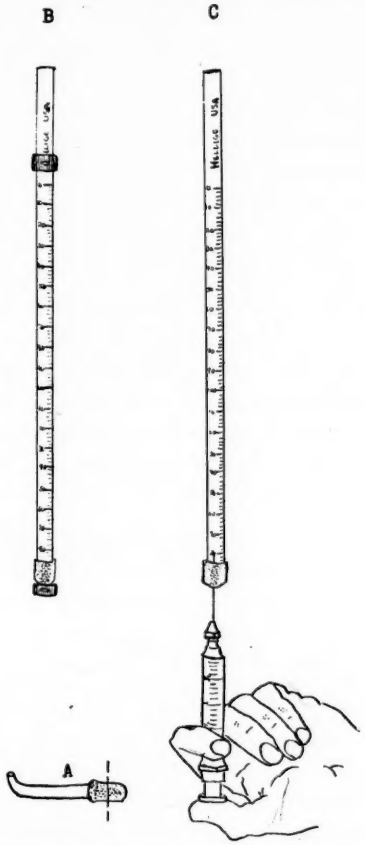

Fig. 1.—*A*, Sample dropper showing line of cut. *B*, Tube held on wall with staples. *C*, Technique of injecting blood into tube.

taking the blood and its introduction into the tube are simplified. The tube is then held on the wall by two staples. The tube rests on the lower staple while the upper one assures the exact vertical position of the tube. The advantage of hanging the tube on the wall in this manner is its simplicity and its safety from any agitation which would invalidate the test. This technique is simple in procedure, costs nothing, and is accurate.

677 Doctors' Building

A MODIFICATION OF THE GRAM STAIN*

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NUMEROUS modifications of the Gram stain have been recommended since the publication of the original method.[1] These attempts can be appreciated for the following reasons: (1) The Gram stain is an important and frequently used procedure; (2) the results vary when the same method but different ingredients are used; (3) the outcome depends to some extent upon the skill and experience of the workers. Some of the more recently used modifications[2, 3] proposed a far more complicated technique in an effort to overcome some previous complaints and handicaps. However, they still retained a few of the previous disadvantages, especially the instability of the ingredients.

I thought it worth while to utilize my experiences with the Gram stain and to devise a procedure which would be more accessible to the average laboratory worker for the daily routine.

Generally one of the most frequently observed difficulties for the ordinary worker represents the time factor of decolorization. Ninety-five per cent alcohol decolorizes too slowly, and acetone decolorizes too rapidly. Probably the next complication in importance for the less experienced worker consists of overstaining with the counterstain. This difficulty can be more easily overcome by using weaker staining solutions for a shorter period of time. The next important obstacle consists of the staining of smears which are not uniformly and usually too thickly spread. In order to decolorize the thicker portions sufficiently, the thinner parts are too extensively decolorized, thus sometimes confusing a proper interpretation of the findings. A too concentrated solution of gentian violet may not keep and might form a sediment; a too weak solution may not produce the desired results. The same observation applies to similar dyes which are occasionally used instead of gentian violet. The ordinary Gram's iodine gives quite different results, depending upon whether the gentian violet: (1) was just poured off, (2) was washed off with water, (3) was washed off with Gram's iodine, or (4) was washed off and dried preceding the application of Gram's iodine. Apparently a prolonged application of gentian violet and Gram's iodine in excess of three minutes has very little additional effect on the results.

Our own experimental work considered the following factors: (1) concentration and stability of the used ingredients; (2) temperature of the solutions at the time of application; (3) application of additional solutions preceding the use of Gram's iodine in order to intensify the staining of gram-positive bacteria; (4) selection of a satisfactory decolorizant; (5) most effective counterstain.

It was found that a 3 per cent solution of gentian violet in a 20 per cent alcoholic solution (gentian violet 3 Gm., 95 per cent alcohol 20 c.c., distilled

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water 80 c.c.) keeps well. Similarly, a twenty times increased concentration of Gram's iodine (20 Gm. of iodine, 40 Gm. of potassium iodine, and 300 c.c. of distilled water) also keeps well and has an increased effect over the ordinary Gram's iodine. Fuchsin was preferred to safranine on account of its bright color. A 2 per cent solution of basic fuchsin in 95 per cent alcohol was found satisfactory, stable, and preferable to other dyes. This stain should be diluted 1:4 with distilled water previous to its use. After considerable experimentation acetone was found to be the most effective decolorizant. Steaming with gentian violet for one minute and subsequent steaming with concentrated Gram's iodine for the same length of time was found slightly preferable to the same procedure without steaming. A series of 10 per cent salt solutions were tried on the smears following the washing off of the gentian violet. A freshly prepared 10 per cent solution of sodium carbonate was found to be very satisfactory; however, the advantage was not sufficiently great to include this step in the proposed procedure.

Consequently the following modification of the Gram stain has been devised: (1) Prepare smears as thinly and uniformly as possible and fix them over the flame. (2) Cover the slide with 3 per cent gentian violet for three to five minutes. (3) Wash with warm water. (4) Cover with twenty times concentrated Gram's iodine for three to five minutes. (5) Wash with warm water. (6) Cover (decolorize) the slide with acetone and wash off immediately with water. (7) Counterstain briefly (on and off) with above dilution of fuchsin. (8) Wash with water, allow to dry, and examine under oil immersion.

DISCUSSION

The proposed modification compares favorably with a number of recently recommended procedures. All the reagents are easily prepared, keep for an indefinite period, and are applied without difficulty. Repeated filtrations and waste of the deteriorated reagents are avoided. Mixing of reagents or other technical steps preceding each individual staining are not required in this technique. The technique of preparing or applying the reagents does not require any particular skill. The differentiation between gram-positive and gram-negative bacteria is very marked. Smears from tissue discharges and those from cultures give equally good results. No sediment is found in the stained preparations. This procedure has been tried out with satisfactory results on a large number of bacteria. The tested bacteria included, among others, various pathogenic and nonpathogenic cocci, intestinal bacteria, diphtheria bacilli, and several anaerobes.

SUMMARY

The proposed modification of the Gram stain offers a number of definite advantages: (1) All reagents keep well. (2) The technique is simple and is easily carried out. (3) The distinction between gram-positive and gram-negative bacteria is very distinct.

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A GLASS-CAPSULE MANOMETER FOR RECORDING THE BLOOD PRESSURE*

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VARIOUS methods of recording the blood pressure continuously in acute experiments in mammals have included the use of glass membranes as in the apparatus of Broemser.[1] The apparatus to be described here has certain advantages, among the more important of which are its sturdiness and sensitivity without the objectionable inertia of the mercury manometer.

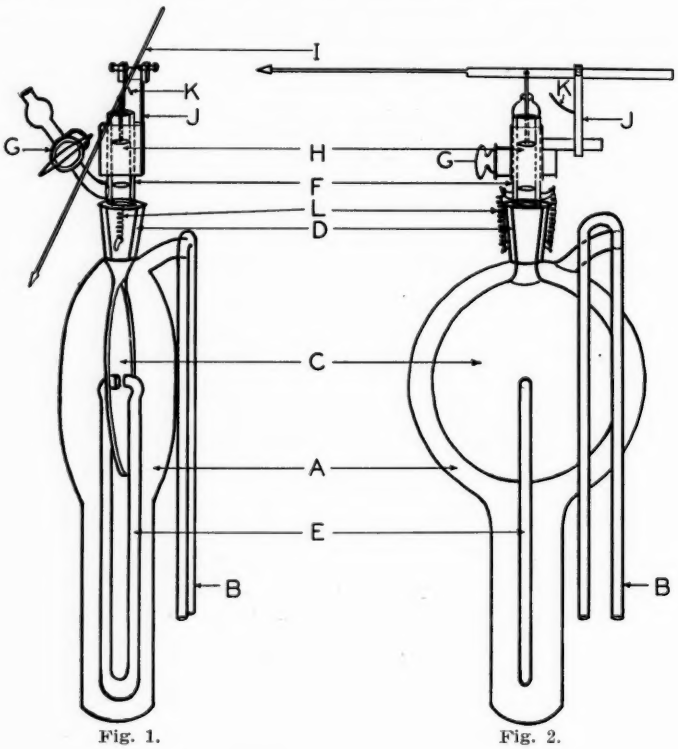

Fig. 1.

Fig. 2.

DESCRIPTION OF APPARATUS

Except for the writing lever and its supports (*I*, *J*, and *K*, Figs. 1 and 2) and the springs, *L*, the apparatus is made entirely of pyrex glass. The heavy-walled chamber *A* is attached to side limbs *B*, so that bubbles may be easily removed. It has been our practice to connect one limb of *B* to a modified Trendelenburg[2] mercury-sodium carbonate pressure system and the other limb to the arterial cannula. The whole system, including chamber *A*, is filled with

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0.1 M sodium carbonate. Capsule *C*, enclosed in chamber *A*, opens into the ground joint *D* and is supported by the U-shaped rod *E*. The glass of the capsule is 0.5 to 0.75 mm. thick. The recording device consists of cylinder *F*, fitting into the ground joint D, the attached stopcock *G*, piston *H*, lever *I*, support *J*, and lever stop *K*. Springs *L* firmly hold the recording device in place.

Capsule *C*, the capacity of which is about 7 c.c., is filled with distilled water. There must be no bubbles of air either in capsule *C* or in chamber *A*. If the apparatus is used under conditions causing rapid evaporation of water, a few drops of watchmaker's oil may be placed on top of the piston. Chamber *A* is filled with 0.1 M sodium carbonate (or other anticoagulant) and connected with the arterial cannula and the Trendelenburg apparatus. Stopcock *G* is opened and the writing lever is pressed down to lever stop *K*. This is the base line. The stopcock is now closed, the stopper is placed in the arterial cannula, and the artery clamp is removed. Ordinarily we have adjusted the apparatus so that a movement of 5 mm. corresponds to a pressure change of approximately 10 mm. of mercury. Exact calibration is, of course, done and accurately reproducible.

The error caused by changes in temperature is small; at approximately room temperature, each change of 1° C. is followed by a pressure change corresponding to 0.8 mm. of mercury.

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A CLOSED-CIRCUIT METABOLISM APPARATUS FOR STUDYING OXYGEN CONSUMPTION OF CONTROL AND THYREOACTIVATOR-TREATED GUINEA PIGS*

HARRY A. TEITELBAUM AND O. G. HARNE, BALTIMORE, MD.

A METHOD that is both simple and rapid was devised to study oxygen consumption in small laboratory animals. The entire outfit can be assembled from apparatus available in most laboratories, thus making it easy to acquire and maintain as many sets as required.

A description of the apparatus and reports of oxygen consumption in control and treated guinea pigs follows:

The metabolism unit, as illustrated in Fig. 1, consists of four fundamental parts: a Mariotte or aspirator flask *a*, a graduated cylinder *e*, an animal chamber *i*, and a weighted stand *l*. With the animal resting in the hermetically sealed chamber on the wire mesh platform *j*, and with appropriate measures taken for the absorption of carbon dioxide by the soda lime *k*, the entire unit is submerged in a constant temperature water bath to the level of the neck of the Mariotte flask. At this time the clamp *c* is closed. In order to allow the ap-

*From the Department of Anatomy, University of Maryland Medical School. The work reported was aided by the Weaver Fellowship Fund.
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paratus to come to the temperature of the bath, an hour is allowed to elapse before readings are started. During this interval a constant current of air is passed through the system by connecting outlet *h* to a suction pump, with clamp *f* open. The opening of the side arm of the graduated cylinder to which clamp *f* is attached must be extended above the surface of the water by means of tubing. It was found advantageous to bubble the air through some water in cylinder *e*, so that the rate of flow could be estimated. The animals were found to rest very quietly throughout this preliminary period using this method.

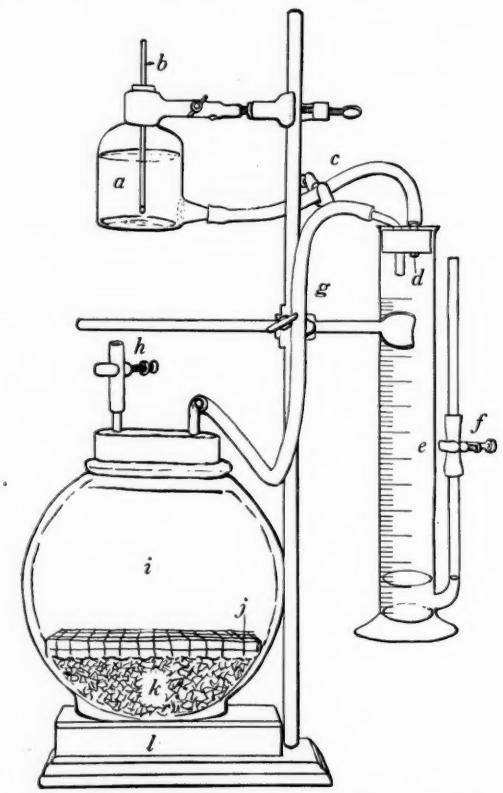

Fig. 1.—Showing the closed-circuit oxygen consumption apparatus, *a*, Mariotte flask; *b*, glass tube; *c*, rubber connection between flask and cylinder; *d*, opening of connection *c*; *e*, graduated cylinder, 200 to 250 c.c.; *f*, side arm on cylinder; *g*, rubber connection between cylinder and animal chamber; *h*, outlet of animal chamber; *i*, animal chamber; *j*, wire mesh platform for animal; *k*, soda line; *l*, weighted stand to keep apparatus submerged.

Before describing the procedure employed in taking a reading, the application of the Mariotte flask *a* deserves comment. This flask must be adjusted at a height that permits the water in the flask to rise in tube *b* about ¼ inch. The water will not flow from the Mariotte flask into the graduated cylinder until the pressure in the cylinder is decreased by the removal of oxygen from the system by the animal within the chamber. With the submerged outlet of tube *b* kept at a constant level, the pressure exerted by the receding height of the water in the Mariotte flask as it flows into the cylinder is kept constant as long as the water level in the Mariotte flask is above the opening of tube *b*. The Mariotte principle functions in such a manner that all the water above the level of the submerged opening of tube *b* serves merely as a reservoir, while the height of

the column of water that exerts pressure in the flowing system is determined by the level at which the atmosphere comes into contact with the water, i.e., at the level of the submerged opening of tube *b*. This avoids the variations that would otherwise be introduced by the gradually decreasing pressure of a diminishing column of water as it flows into the cylinder from the flask.

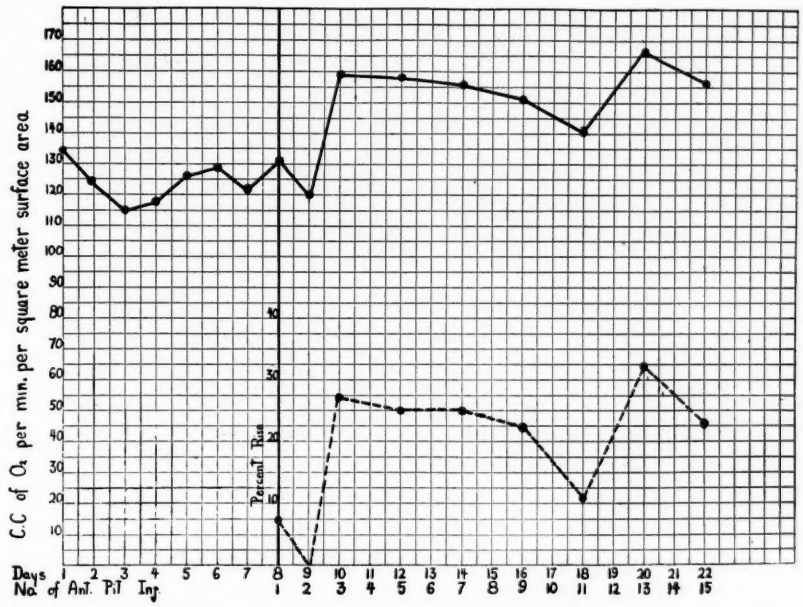

Fig. 2.—Graph showing the effect of daily doses of thyreoactivator hormone derived from 25 mg. of dried beef anterior lobe powder on the oxygen consumption of a guinea pig.

When ready to start a reading, the air or oxygen stream is stopped. Clamps *f* and *h* are closed, and clamp *c* is opened. When the animal has exhausted enough oxygen to lower the pressure inside the system, the water begins to flow from the Mariotte flask into the cylinder. After the flow has started, the height of the water in the cylinder is recorded and readings thereafter are made at any desired interval. The amount of water that flows into the cylinder is equivalent to the volume of oxygen consumed by the animal.

When the experiment is completed, clamp *c* is closed again, and clamps *f* and *h* are opened. The water is evacuated from the cylinder by applying suction to the side arm *f*, while tube *h* is open to the atmosphere. The water in the Mariotte flask is replenished from the water bath, and the oxygen supply is restored by a current of air as described. The readings can thus be repeated any number of times.

Theoretically barometric pressure corrections should be made for each reading. However, the barometric pressure changes are so slight in the case of readings of such short duration that they were found to be of very little significance in an apparatus of such large volume as that used for the guinea pigs. In studies of the oxygen consumption of salamanders using the principle described, with appropriate modifications to suit the size of the animal, Thompson and Uhlenhuth (1936) found that barometric pressure corrections were essen-

tial. Because of the small quantities of oxygen consumed, it was found desirable to take readings over six- to eight-hour periods, during which time the barometric pressure could change significantly.

An example of the results obtained with the apparatus described is shown in Fig. 2. Here the effect of daily injections of an extract derived from 25 mg. of dried anterior lobe powder (beef) on the oxygen consumption of a guinea pig is represented.

TABLE I

OXYGEN CONSUMPTION OF 15 YOUNG GUINEA PIGS

(Each value is an average of at least five readings taken on separate days.)

WEIGHT, GM.	162	145	156	132	137	160	159	146	172	150	150	172	115	147	167
SEX	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
C.C. OXYGEN SQ.M. PER MIN.	123	139	138	131	137	146	129	141	139	130	135	135	134	139	139

Table I gives records of 15 untreated guinea pigs of both sexes ranging from 115 to 172 Gm. in weight. The oxygen values are recorded in cubic centimeters per square meter body surface per minute. The experiments were done in a constant temperature bath at 31° C. Friedgood (1934), using a modified Benedict closed circuit apparatus at 32° C., obtained average oxygen values in cubic centimeters per square meter per minute of 105 c.c. for male and 99 c.c. for females. His guinea pigs, however, weighed between 400 and 650 Gm. The higher values revealed in Table I are undoubtedly due to the younger age of the animals. DuBois (1927), discussing the effect of age on metabolism, pointed out the inverse ratio existing between the two during the early periods of rapid growth.

SUMMARY

1. A closed-circuit apparatus for the study of oxygen consumption in small laboratory animals has been described. This apparatus has the advantages of simplicity and rapidity. It has been used satisfactorily with the guinea pig, rat (Krantz and others), and salamander.

2. Oxygen consumption of a guinea pig treated with thyreoactivator hormone is shown to be increased (Fig. 2).

3. Oxygen consumption of a colony of 15 young guinea pigs is shown to be comparable. No certain sex variations were observed (Table I).

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A MODIFIED TUBE FOR TATTOO MACHINES*

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TATTOO has been employed in medical practice for the correction of cosmetic defects of the skin[1] and eye, and for the marking of laboratory animals. I introduced tattoo with mercuric sulfide for its therapeutic effect in pruritus ani.[2, 3]

In tattoo an insoluble chemical is deposited through the epidermis into the corium by means of one or more needles. The standard machine consists of an armature (*L*) which vibrates through the magnetic action of two electromagnetic coils (*M*). A needle bar (*N*) attached to the armature passes through the tube (*B*). Six to 8 needles connected to the distal end of the bar move forward and backward with a thrust of 2 mm. by the vibration of the armature. The tube is held and moved like a writing pencil, with sufficient pressure to insure the penetration of the needles into the corium without lacerating the skin.

The purpose of the treatment of pruritus ani by tattoo is to deposit mercuric sulfide uniformly throughout the corium of the involved skin. When this is done, a uniform deep red tattoo results; the itching is relieved and the skin returns to its normal texture. To obtain a uniform tattoo, I use four upstrokes with each application of the chemical. The tube is held at an angle of 30 to 45 degrees to the skin. The corium is penetrated when the needles are kept in close contact with the skin and when sufficient current is used to make the armature vibrate freely against the resistance of the skin. It is essential that the chemical be always present at the needle points, and that these points lie parallel to the surface of the skin.

The following tube has been devised to insure the presence of a chemical at the needle points of a tattoo machine and to reduce the tension on the finger that directs the needles. It consists of a piece of hollow brass (*A*) ¼ inch in diameter and 1¼ inches in length. It is split lengthwise on its upper surface and is soldered to the tube (*B*) ¼ inch from its tip.

The distal end of the brass piece (*C*) is flattened to equal the width of the needles, and projects ⅛ inch forward from the soldered base. When the tube is dipped into the chemical used for tattooing, this end acts as a reservoir to feed the needles. It is then practicable to increase the number of needles to the limit of the diameter of the tube, which is 18 needles. By increasing the number of needles from the customary 6 to 8, the time required to tattoo a certain area of skin is reduced.

Where the skin is thin, it is practicable to use 18 needles to produce a uniform deposit of the chemical throughout the corium, resulting in a uniform

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deep red tattoo. However, where the skin is hyperkeratotic or leathery, not more than 8 needles should be used. I have found that the resistance of such epidermis is too great for the uniform penetration of more than 8 needles.

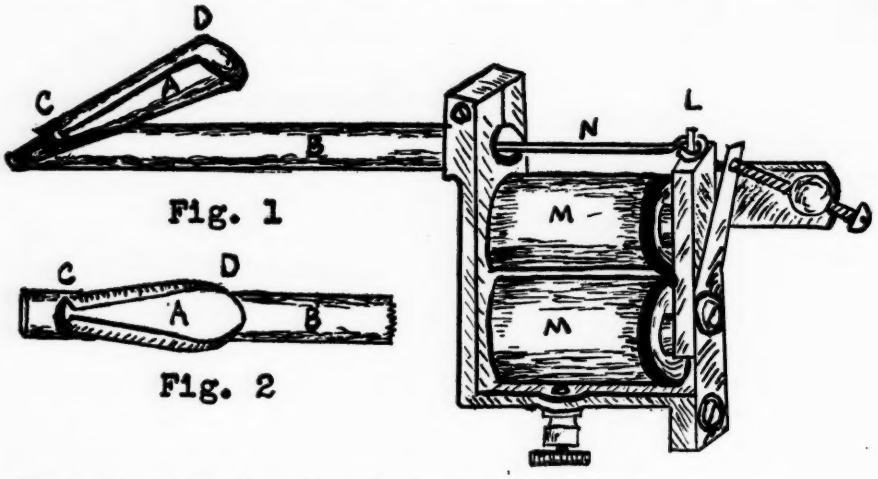

Fig. 1

Fig. 2

Fig. 1.—View of the tube and machine from the side and front.
Fig. 2.—View of the tube from the top and front.

The proximal end of the attachment (*D*) is opened into a smooth flange on which the thumb or index finger rests. This acts as a fulcrum to balance the weight of the machine (8 ounces) which reduces the pressure that the finger must exert to direct the needles on the skin. Preventing fatigue of the fingers is especially important in tattooing an irregular surface, such as the perianal region, for the direction of the needles must be changed frequently to keep their points parallel to the plane of the skin.

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CHEMICAL

THE ALCOHOL OF THE LUNG AIR AS AN INDEX OF ALCOHOL IN THE BLOOD*

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THE concentration of alcohol in the blood is now frequently determined from the concentration of alcohol in the air of the lungs. The values so obtained are used, not only as evidence of intoxication for the police court, but also as the basis for studies in regard to the drinking habits of motorists.

It is certain that the concentration of alcohol in the lung air varies directly with that in the blood. But there are differences of opinion as to the correct figure for the coefficient of distribution; that is, the figure by which the amount of alcohol in 1 c.c. of air should be multiplied to give the amount in 1 c.c. of blood. There is also a difference of opinion as to the procedure for determining the concentration of alcohol in the lung air. Uniform results are not obtained with the various methods now in use. This paper presents a critical study on both points.

The results obtained indicate that the coefficient now commonly employed is incorrect,[1] that the method now chiefly used for collecting lung air[1] does not give a correct value for the alveolar concentration of alcohol, and that consequently the results obtained for concentration in the blood and employed for juristic and statistical purposes may be correspondingly erroneous.

In 1934 two of us (H. W. H. and L. A. G.[2]) determined the coefficient of distribution of alcohol between air and water as 1:1,456 at 37.5° C., which we took as the temperature at which the distribution occurred in the lungs; we determined the coefficient for air and blood at the same temperature as 1:1,150. Harger, Lamb, and Hulpieu,[1] for an apparatus called the "drunkometer," chose a value of 2,000 for the coefficient of distribution of alcohol between air and blood. Haggard, Greenberg, and Cohen[3] in turn criticized the accuracy of this procedure, both because of the high coefficient and because expired air was collected in a rubber bag. They were certain, from their own experience, that such collection led to the loss of considerable quantities of alcohol, and were of the opinion, but incorrectly as is here shown, that the loss was primarily by diffusion. In studies in which alveolar air was used in determining the concentration of alcohol in the blood, they were forced to employ trained persons who exhaled true alveolar air directly into the train of the analyzer.[3] At that time this was the only method by which they were able to

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obtain, with the low coefficient which they employed, correct values for the concentration of alcohol in the blood as shown by direct determination from the blood.

In the course of the present study we have uncovered a feature which has not been previously taken into consideration in determining the concentration of alcohol in the blood from the concentration in the lung air, and which affords, in part at least, an explanation for the discrepancies between the coefficient used by two of us (H. W. H. and L. A. G.) and the one used by Harger, Lamb, and Hulpieu.[1] It is the retention of alcohol in the film of moisture which condenses on the surfaces of tubes and containers used in collecting expired air. When expired air at body temperature is cooled to room temperature in any container, water vapor condenses immediately as a mist, the particles of which rapidly coalesce to form a film on the inner surface of the container. The particles of the mist provide a large surface which permits a rapid equilibrium between the water and the alcohol vapor in the air. This equilibrium is established at a lower temperature, and, therefore, at a higher coefficient of distribution, than at body temperature. Although the total amount of water separated from the air is small, the high solubility of alcohol in water causes a marked reduction in the amount of alcohol in the air from which it condenses. The concentration in the air, as determined by analysis, is correspondingly lower than that which was actually present in the air before it left the lungs.

Since this feature, which must be considered in handling alcohol vapor, was unrecognized by two of us (H. W. H. and L. A. G.[2]) at the time we determined the coefficient distribution of alcohol between air and water and air and blood,[2] these values have here been redetermined over a range of temperature from 20° to 40° C.

1. COEFFICIENT OF DISTRIBUTION OF ALCOHOL BETWEEN AIR AND WATER

The apparatus used in determining the coefficient of distribution of alcohol between air and water is shown in Fig. 1*A*. A stream of air flowing at the rate of 500 c.c. per minute, as determined by the calibrated manometer *M*, is washed through a saturated dichromate solution in concentrated sulfuric acid and passed successively through flasks *A*, *B*, *C*, and *D*, each containing 2 liters of alcohol solution of approximately 1 Gm. per liter. Dispersion of the air at the outlets in the flasks is obtained with fine carborundum bubblers. The flasks, with all connections and stopcocks, are completely immersed in a water bath held at the desired temperature. The syringes, *E* and *F*, mounted on flasks *C* and *D*, permit withdrawal of water to determine the alcohol content by analysis. Flask *D* is open to the room through tube *G* which has a large bore; the pressure of air in flask *D* is not measurably above that of the room during the flow of air. The pressure in flask *C* can similarly be reduced by opening stopcock *I* when samples of air or water are withdrawn for analysis.

In determining the content of alcohol in the air of flask *D*, a measured volume of air at the rate of approximately 100 c.c. per minute is drawn through stopcocks *H* and *O* through an iodine pentoxide analyzer train.[4] The volume of air passed through the analyzer is measured at atmospheric pressure and room temperature, but is corrected to the temperature in flask *D*.

An important feature of the apparatus shown in Fig. 1 is the provision made for evaporating condensed water vapor from the capillary tubes leading to the analyzer train. The condensate which forms, when the temperature of the bath is higher than that of the room, may hold considerable quantities of alcohol. After the desired amount of air for analysis is drawn through stopcock *H*, this stopcock is closed and stopcock *O* is turned so that a stream of fresh air will pass through the tube to the analyzer train. Any water which has condensed is evaporated and, together with the alcohol which it holds, is carried into the analyzer.

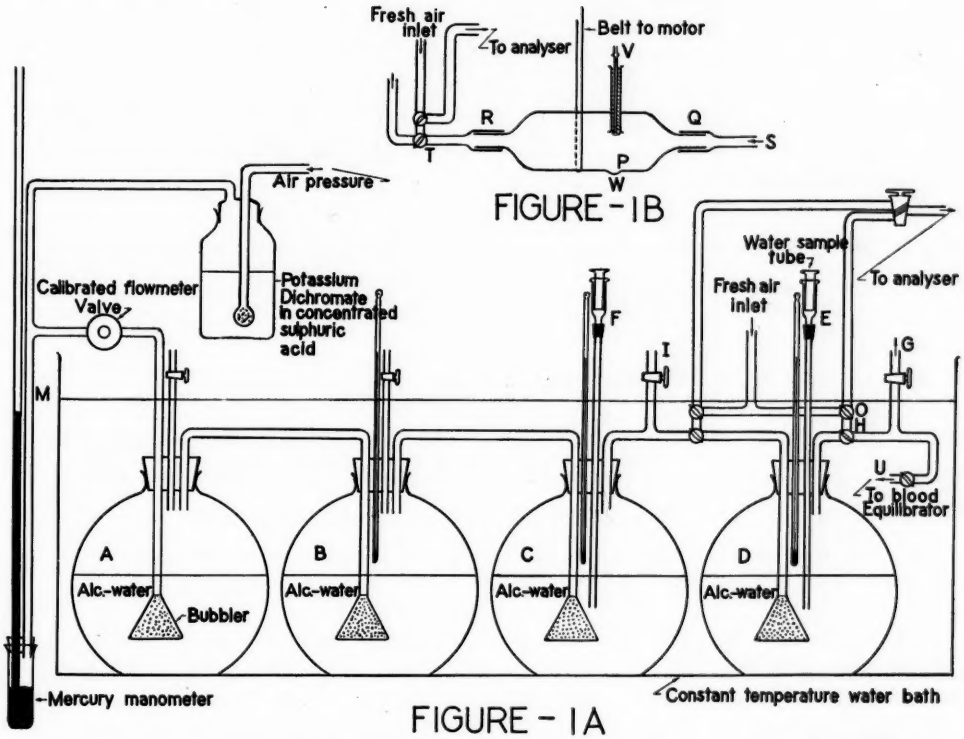

Fig. 1.—Apparatus for determining distribution of alcohol between air and fluids.

Completeness of equilibrium of alcohol between air and water in flask *D* was taken to exist when the concentration of alcohol in the air of flask *C*, as shown by analysis, corresponded exactly with that from flask D. In determining the coefficient of distribution at any temperature, the concentration of alcohol in the air of flask *D* is determined, and simultaneously water is withdrawn with syringe *E* and measured amounts are analyzed for alcohol. The calculation of the ratio is made as

$$(1) \qquad 1: \frac{A' V}{A V'},$$

in which *A* is the amount of alcohol in milligrams in the air drawn through the analyzer train; *V*, the volume of air in cubic centimeters corrected to the temperature in flask *D;* and *A'*, the amount of alcohol in milligrams in the volume

of water, V', in cubic centimeters, taken for analysis. Fig. 2 gives the values obtained for temperatures from 16.5° to 40° C.

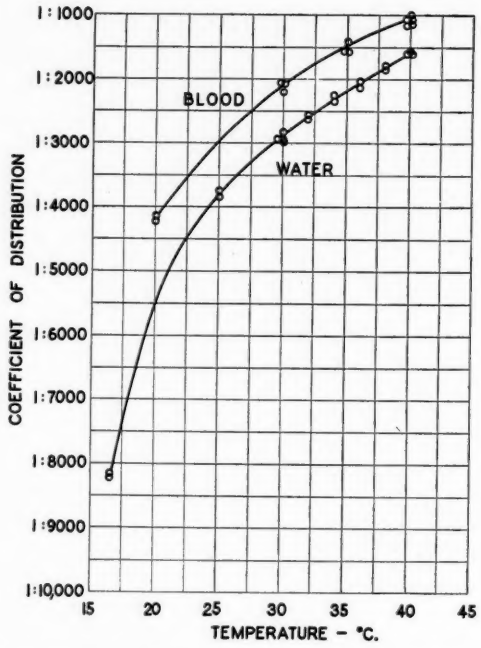

Fig. 2.—The distribution of alcohol between air and water, and between air and blood. Concentration of alcohol 0.06 to 0.30 per cent.

It should be possible to calculate the coefficient of distribution of alcohol between air and water from the values given in the literature for the vapor pressure of alcohol over mixtures of alcohol and water. These determinations have, however, been made with mixtures containing 10 per cent or more of alcohol by weight. The concentration found in the blood rarely exceeds 0.5 per cent. At temperatures as high at 40° C. the coefficient of distribution is considerably affected by such changes in concentration as that between 0.5 and 10 or 20 per cent. The coefficient of distribution is calculated from the vapor pressure from the ratio:

$$(2) \qquad 1: \frac{10\ \mathrm{Pd} \times 76 \times 22.4\ (273 + t)}{46\ \mathrm{pp} \times 273},$$

in which P is the per cent by weight of alcohol in the mixture; d the density of the mixture at temperature t; and pp the partial pressure of the alcohol vapor over the mixture at this temperature. At 39.76° C., Wrewsky[5] gives the vapor pressure of alcohol over water containing 42 per cent by weight of alcohol as 63.0 mm.; 22 per cent as 44.55 mm.; 18.25 per cent as 40.1 mm.; and 15.92 per cent as 37.1 mm. The coefficients of distribution as calculated from these values are 1:2,590, 1:2,000, 1:1,865, and 1:1,760, respectively. From three determinations made at 40° C. on water containing 0.01 per cent of alcohol, we find coefficients (Fig. 2) of 1,580, 1,631, and 1,638.

2. COEFFICIENT OF DISTRIBUTION OF ALCOHOL BETWEEN AIR AND BLOOD

In determining the distribution of alcohol between air and blood, the apparatus shown in Fig. 1*B* is used in addition to that shown in Fig. 1*A*. The entire apparatus is immersed in the water bath of apparatus in Fig. 1*A*. Bulb *P* is of 100 c.c. capacity and is fitted by long ground joints, *Q* and *R*, to tubes *S* and *T*. Tube *S* is connected under water to tube *U* of apparatus *1A*, and a stream of air containing alcohol vapor from the flasks *A* to *D* is passed through bulb *B* at the rate of 750 c.c. per minute and discharged through tube *T*. Two cubic centimeters of blood are delivered into the bulb through tube *V* which projects to the middle of the bulb and is closed by a glass-tipped stopper. The bulb is rotated at the rate of 10 to 15 r.p.m.; the blood is thus spread in a thin film on the wall of the bulb for equilibration with the air containing alcohol.

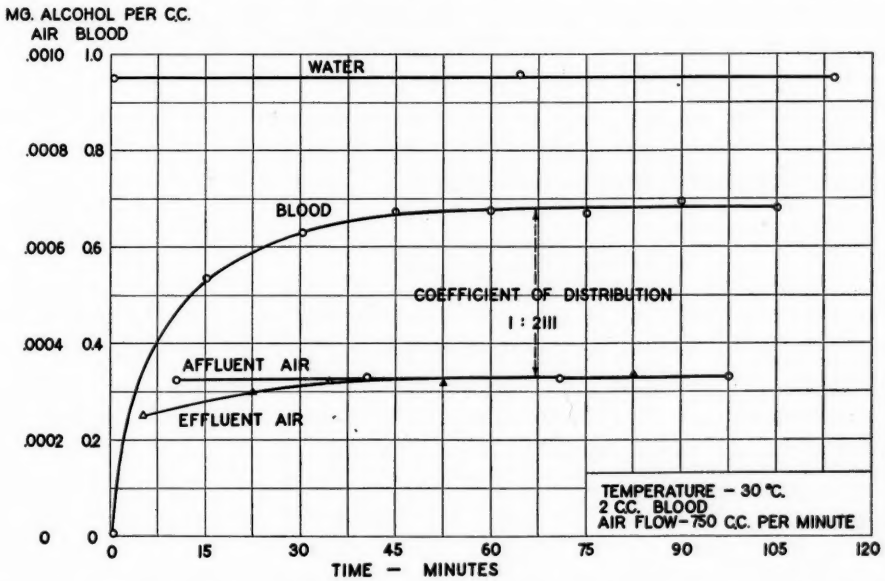

Fig. 3.—The concentrations of alcohol in air and blood during equilibration in apparatus shown in Fig. 1*A* and *B*.

There is no evaporation of the blood since the air-leaving flask *D* is fully saturated with water at the temperature of the bath. In withdrawing blood for analysis, the rotation of the bulb is stopped with tube *V* in a vertical position, as shown, and projecting above the level of the water in the bath. Blood collects in the depression *W* and is withdrawn with a pipette inserted through tube *V*. Affluent air of the bulb is obtained for analysis through stopcock *H* of apparatus in Fig. 1*A* and effluent air through stopcock *T*. Precautions are taken, as described, to remove condensed water vapor.

In determining the distribution of alcohol between air and blood, the flow of air was continued for ninety to one hundred and twenty minutes. In order to be certain that equilibrium was complete, analyses were made at intervals of the alcohol in the water of flask *D*, in the affluent and effluent airs of bulb *P*, and in the blood. The values obtained in one such determination are shown in Fig. 3. Equilibration was complete here in sixty minutes. As a further

check upon the accuracy of the method used, a series of determinations was made with water instead of blood; the values obtained corresponded exactly with those determined as described in the previous section and given as the curve for water in Fig. 2.

Fig. 2 gives the values obtained for the coefficient of distribution of alcohol between air and blood at temperatures of 20° to 40° C. The values at 35° and 40° C. are 1:1,550 and 1:1,124, respectively. The values previously determined by two of us (H. W. H. and L. A. G.[2]) for these temperatures are 1:1,204 and 1:1,130. The value of 1:2,000, employed by Harger, Lamb, and Hulpieu[1] corresponds, from our determinations here, to a temperature of 31° C.

This value of 1:2,000 was not determined in vitro by Harger, Lamb, and Hulpieu, but is that given by Liljestrand and Linde.[6] These latter investigators obtained the value empirically from determinations made on expired air and blood. No consideration was given to the loss of alcohol in condensed water vapor in collecting the air. Moreover, they are of the opinion that mixed expired air and alveolar air contain the same amounts of alcohol, a feature which Harger, Lamb, and Hulpieu were unwilling to accept, although they employed their coefficient of distribution.

3. CONCENTRATION OF ALCOHOL IN THE ALVEOLAR AND VENOUS AIRS

It is difficult to obtain on untrained subjects, particularly if they are partially intoxicated, a reliable sample of alveolar air. For these reasons, Harger, Lamb, and Hulpieu[1] collected mixed expired air, determined both the concentration of alcohol and the per cent of carbon dioxide, and converted the former to what they assumed to be the alveolar concentration of alcohol on the basis of a constant 5.5 per cent of carbon dioxide in alveolar air. This procedure involves the assumption that carbon dioxide and alcohol diffuse with equal rapidity into the air of the respiratory dead space, an assumption which we shall later demonstrate to be incorrect. In obtaining the alveolar concentration of alcohol we[3] have previously employed trained subjects and taken, as alveolar air, the last third of expiration as in the method of Haldane and Priestley.[7] We find now that this procedure is unnecessary and that the venous air[8] obtained by a short period of rebreathing gives, for both trained and untrained subjects, a concentration of alcohol that is nearly identical with that obtained from alveolar air. These same relations have been shown previously to hold for carbon monoxide present in the blood.[9]

A consideration of the principles of elimination of alcohol shows the theoretical validity of the procedure. Because of its high solubility, alcohol is eliminated only in small amounts in the expired air; the concentrations in the pulmonary venous and arterial bloods, unlike the concentrations of carbon dioxide and oxygen, are only slightly different. The alcohol in the blood reaching the lungs is distributed between the respective volumes of air and blood in the relation of the distribution coefficient.[10, 11] Therefore,

$$(3)\quad Ac = \frac{B + \frac{L}{C}}{Ve\ B},$$

in which *Ac* and *Vc* are the concentrations of alcohol in grams per liter in the arterial blood leaving the lungs and the venous blood reaching the lungs; *B*, the volume of blood circulated through the lungs in time *t*; *L*, the volume of pulmonary ventilation in this time; and *C*, the coefficient of distribution of alcohol between blood and air with the concentration in air taken as 1.

During rest the volume of the circulation through the lungs and the volume of the pulmonary ventilation are approximately the same. On this basis, the relation between the concentrations of alcohol in the arterial and venous bloods at a distribution coefficient of 1:1,300 would be 1:1.00077 (equation 3). The error introduced in the determination of the arterial concentration would, therefore, be 0.077 per cent. Even wide variations in blood flow and volume of pulmonary ventilation would not introduce an appreciable error; thus if the ventilation were ten times as great as the blood flow, the theoretical error as calculated would be increased from 0.077 to only 0.77 per cent.

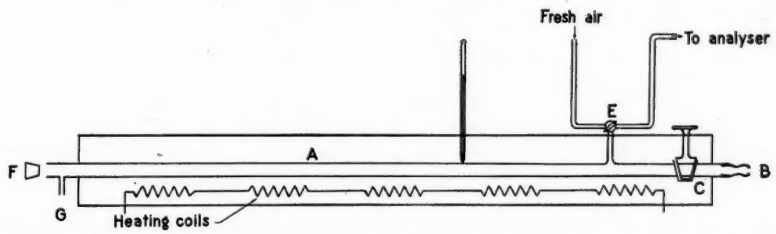

Fig. 4.—Apparatus for determining the concentration of alcohol in the alveolar and venous airs.

The apparatus shown in Fig. 4 is used to determine the concentration of alcohol in both alveolar and venous airs. It consists of a glass tube, *A*, of 2.5 cm. bore, 2 meters long, contained in an asbestos lined wooden box, 15 cm. in sectional diameter. The tube projects a short distance from the ends of the box and is fitted at one end with a glass mouthpiece, *B*, made tight with a ground joint and removable for sterilization with dry heat. The stopcock *C*, of 1.25 cm. bore, is controlled from outside the box and serves to close off the tube after the sample of lung air has been collected. To prevent condensation of water vapor with loss of alcohol, as discussed in the next section of this paper, the tube is kept at a temperature slightly above 40° C. by heating coils; the current is regulated by a rheostat.

Alveolar air can be collected in the tube as in the procedure described by Haldane and Priestley.[7] Following a normal inspiration, a deep expiration is made and stopcock *C* is closed. Venous air is collected by having the subject wear a nose clip and rebreathe from the tube six times; stopcock *C* is closed at the end of the last expiration. To determine the concentration of alcohol in the air collected, stopcock *E* is opened and 100 to 200 c.c. of air are drawn from the tube into the analyzer train by water displaced as described for the apparatus shown in Fig. 1*A*; suitable correction is made for the difference in temperature of the air in the tube and that in the room. Stopcock *E* is then turned to permit a flow of fresh air, obtained outside the room, to flush the tubes leading to the analyzer train and to remove any condensed water vapor. The expired air is flushed from tube *A* by opening stopcock *C*, inserting stopper *F*, and drawing

a brisk current of air through tube *G* which is connected to a suction pump. The apparatus described here can be assembled in more compact form by having tube *A* bent into a coil and enclosed in a square box.

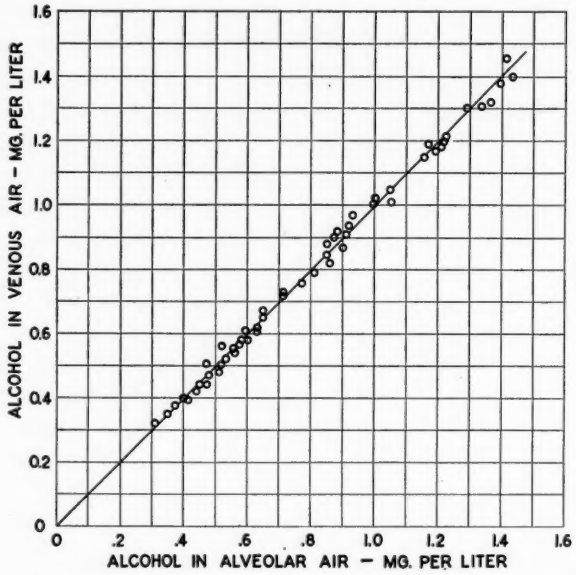

Fig. 5.—Correlation between the concentration of alcohol in alveolar and venous airs.

Fifty-three pairs of comparative determinations were made of the concentrations of alcohol in the alveolar and venous airs of 5 trained subjects. They each drank from 30 to 150 c.c. of alcohol diluted to 50 per cent by volume with water. At intervals thereafter the concentration of alcohol in the alveolar air was determined and immediately following, that in the venous air. Fig. 5 presents the values obtained; each dot is made at the point of intersection of lines drawn from the ordinate value for the concentration in the venous air and from the abscissa value for that in the alveolar air of corresponding determinations. The average of all the values obtained for the alveolar air was 0.814, and for the venous air, 0.823 mg. per liter. The maximum variation in any single pair of determinations was less than 5 per cent.

4. CONCENTRATION OF ALCOHOL IN THE MIXED EXPIRED AIR

It is possible, as is demonstrated in the next section of this paper, to collect expired air in a rubber bag without appreciable loss of alcohol during the first five minutes, provided the bag is kept warmed to a temperature of 40° C. to prevent condensation. In the study here a rubber bag was placed in an incubator kept slightly above this temperature; the short glass tube leading to the bag, and used as a mouthpiece by the subject, was passed through the side of the incubator and was warmed with a small heating coil. In obtaining mixed expired air the subject, previously given alcohol, exhaled into the bag; the bag was emptied and a second expiration was collected. The mouthpiece was then closed and a measured sample of the air was drawn into the analyzer train with precautions, as previously described, to remove the condensed water vapor.

Table I gives a series of values obtained from one subject for the concentration of alcohol and per cent of carbon dioxide in the mixed expired and alveolar airs. Nine similar series made with this subject and with four other subjects gave the same results within the ordinary limits of individual variation.

TABLE I

SUBJECT GIVEN BY STOMACH 110 C.C. OF ETHYL ALCOHOL DILUTED TO 50 PER CENT WITH WATER

TIME AFTER GIVING ALCOHOL (HR.)	ALCOHOL		CARBON DIOXIDE		
	MIXED EXPIRED AIR (MG./L.)	ALVEOLAR AIR (MG./L.)	EXPIRED AIR (%)	ALVEOLAR AIR (%)	ALCOHOL IN MIXED EXPIRED AIR $\times \frac{CO_2 \text{ ALVEOLAR}}{CO_2 \text{ EXPIRED}}$ (MG./L.)
1	1.01	1.15	4.1	5.6	1.38
2	0.97	1.15	3.8	5.5	1.40
3	0.85	1.06	3.9	5.8	1.26
4	0.71	0.90	4.0	5.6	0.99
5	0.63	0.77	3.9	5.8	0.93
6	0.48	0.61	3.7	5.4	0.70
7	0.40	0.45	3.9	5.6	0.58

The last column of Table I gives the concentration of alcohol calculated for alveolar air as in the procedure followed by Harger, Lamb, and Hulpieu,[1] i.e., concentration of alcohol in mixed expired air $\times \frac{CO_2 \text{ in alveolar air}}{CO_2 \text{ in mixed expired air}}$. In each instance this calculated value is higher than that obtained by direct measurement: the average difference is 18 per cent, and the minimum and maximum differences are 9 and 23 per cent. These findings suggest that alcohol diffuses more rapidly from the surface of the respiratory passages into the air in the respiratory dead space than does carbon dioxide. The diffusion of alcohol from these surfaces does not affect the concentration of alcohol obtained directly from the alveolar or venous air, but only from the mixed expired air, approximately one-third the volume of which comes from the virtual dead space. Haldane[12] has demonstrated that the carbon dioxide which diffuses from the respiratory passages enters the mixed expired air, but not the exhaled alveolar air. In rebreathing, as in the procedure used here to obtain venous air, equilibrium of alcohol is reached throughout the entire respiratory tract. We have demonstrated that the concentrations of alcohol in the alveolar and venous airs are nearly identical (Fig. 5).

The extensive diffusion of alcohol into the air in the respiratory dead space has been previously observed by Liljestrand and Linde;[6] they found that mixed expired air contains nearly as much alcohol as the alveolar air. As seen from the values given in Table I, the concentration of alcohol in the alveolar air is, however, higher than in the mixed expired air. The average difference is 21 per cent.

A series of experiments was carried out here to demonstrate that the diffusion of alcohol into the air of the respiratory dead space is more rapid than that of carbon dioxide. For this purpose the mouth air was used, as in the studies of Henderson and Stehle[13] on the diffusion of carbon dioxide and oxygen. A subject, previously given alcohol, inhaled and then immediately closed off the

mouth at the pharynx and breathed through the nose. A glass tube was held between the lips, and the mouth was inflated with 75 c.c. of air by means of a hand bulb attached to the tube; the mouth was then emptied and reinflated. The air was held for two minutes, and the concentration of alcohol and the per cent of carbon dioxide in it were then determined. Immediately thereafter the concentration of alcohol and per cent of carbon dioxide in the alveolar air were determined. In a series of four such experiments the concentrations of alcohol in the mouth air were 42.4, 43.2, 41.7, and 44.9 per cent of that in the alveolar air; the per cents of carbon dioxide in the mouth air were only 16.3, 14.6, 15.1, and 17.2 of that in the alveolar air. In a second series of experiments, in which the air was held five minutes instead of two, the differences in rate of diffusion were still evident, although less marked; the average value for the concentration of alcohol was 46.2 per cent, and the carbon dioxide was 36.1 per cent of that in the alveolar air.

The concentration of alcohol in the mixed expired air is less than that in the alveolar air by approximately the same amount that it is exceeded by the concentration calculated for the alveolar on the basis of the per cents of carbon dioxide (columns 2, 3, and 6, Table I). It would appear then to make little difference, so far as accuracy is concerned, which of the two values is taken in calculating the concentration of alcohol in the blood; the error would be the same, but in opposite directions. An error of 20 per cent in calculating the concentration of alcohol in the blood from that in the lung air is probably not serious if such determinations are intended only as rough clinical approximations or are to be used, as they often have been used, in police tests to coerce the suspect into a confession of having drunk immoderate amounts of alcohol.[1, 14]

This, however, is not the only error resulting from the procedure as now generally used. A further and widely variable error is introduced by this method of collecting the expired air and will be discussed in the following section.

5. LOSS OF ALCOHOL IN CONDENSED WATER VAPOR

When expired air is collected in containers or passed through tubes at a temperature lower than that of the body, alcohol, as we have stated, is removed in the condensed water vapor. This loss, and the corresponding error introduced into determinations of the concentration of alcohol in respired air, is not constant, but varies with the temperature of the container. The concentration of carbon dioxide in the air, because of the low solubility of this gas, is not appreciably affected by the condensation of water vapor.

The following experiments demonstrate the loss of alcohol in condensed water vapor. Air at 40° C. containing alcohol and saturated with water vapor was run from outlet *G* of flask *D*, of the apparatus shown in Fig. 1*A*, into a rubber bag. The bag was placed in an incubator so that it could be kept at any temperature desired. The short glass connection between the source of alcohol vapor and the bag was warmed with an electric heating coil. After 1 liter of air had been collected, the bag was emptied and again filled with this quantity of air. Samples of air were drawn from the bag at two, five, fifteen, and thirty minutes, and the concentration of alcohol was determined. The values obtained are shown in Fig. 6.

When the bag in which the air was collected was warmed to 40° C. (curve A, Fig. 6), the loss of alcohol was slow and nearly uniform; in five minutes the concentration had fallen 3 per cent, and in thirty minutes, 13 per cent. When the bag was kept at a temperature of 20° C. (curve C, Fig. 6), there was an immediate sharp drop in concentration, followed by a slow progressive drop; in two minutes the concentration had fallen 18 per cent; in five minutes, 26 per cent; in fifteen minutes, 30 per cent; and in thirty minutes, 34 per cent. When the bag was cooled to 10° C. (curve D, Fig. 6), the decreases in concentration at

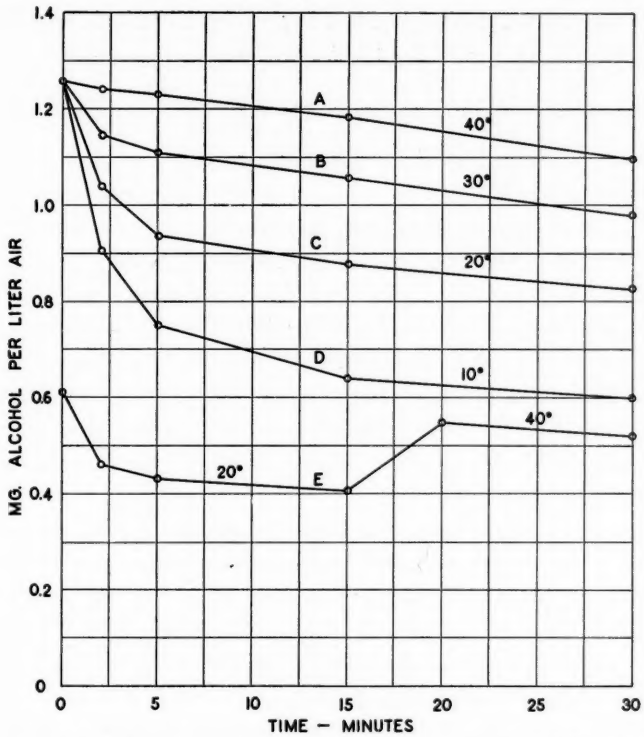

Fig. 6.—Loss of alcohol from lung air in condensed water vapor at different temperatures.

the corresponding times were 28, 41, 50, and 52 per cent. In a final series of determinations (curve E, Fig. 6), the bag was first kept at 20° C. for fifteen minutes; during this time the concentration fell 32 per cent. The bag was then warmed to 40° C. to evaporate the condensed water vapor; the concentration of alcohol rose, reaching a value only 14 per cent below the initial concentration.

From the results obtained here it is evident that if expired air is collected, as is now the practice,[1] errors as great as, or even greater than, 50 per cent are to be expected. Such a collection is made from motorists at the roadside, under a wide range of daily and seasonal temperatures, and no precautions are taken to prevent condensation of water vapor from the breath. The error from condensation can be avoided only by having the receptacle in which the air is collected at a temperature sufficiently high to prevent condensation of moisture, as is done in the apparatus shown in Fig. 4.

Loss of alcohol in condensed water vapor occurs, not only when rubber bags are used to collect expired air, but also when the air is passed through glass or

metal tubes. This fact is demonstrated here by a series of determinations of the concentration of alcohol in alveolar air (Haldane and Priestley) made with the apparatus shown in Fig. 4. In separate determinations the tube was kept at 10°, 20°, 40°, and 50° C. One trained subject was used and ten determinations were made at each temperature. The coefficient of distribution of alcohol between air and blood was calculated from the concentration of alcohol obtained in the alveolar air and that in blood drawn simultaneously. At 10° C. the average value found for the coefficient was 3,123; at 20° C., 1,819; and at 40° C., 1,304. On increasing the temperature of the tube to 50° C., no further change occurred in the coefficient, which at this temperature was 1,306.

6. COEFFICIENT OF DISTRIBUTION AS DETERMINED ON 100 SUBJECTS

One hundred different subjects were either given alcohol or were brought to the laboratory after they had taken alcohol. No comparison between the concentration of alcohol in the blood and venous air was made until it was certain, from repeated determinations on the blood, that the concentration of alcohol had reached its maximum and had decreased over a period of at least one hour. This feature is important in using the concentration of alcohol in the capillary or venous blood as an index of that in the arterial blood. When absorption and distribution of alcohol are complete, the concentration of alcohol in blood from all sources corresponds closely, but prior to this time, exact agreement does not exist and appreciable errors may be introduced into the calculation of the distribution coefficient.[2, 15]

The blood of the subjects was tested for acetone by the method described by Shipley and Long;[16] no appreciable amount was found in any instance. This procedure was followed, since iodine pentoxide, used for the analysis of alcohol, reacts to acetone. In the analysis of blood the acetone can be removed with Mariott Scott-Wilson reagent,[11] but similar removal cannot be made from the respired air. In determinations made on more than 200 subjects given alcohol, we have found no amount of acetone in the blood sufficient to introduce any error into the determination of alcohol. An equal number of determinations made on the expired air of these subjects before they were given alcohol showed a similar absence of oxidizable material when the sample taken for analysis was not larger than 200 c.c. This result was obtained even on subjects who had eaten onions or candy containing essential oils. From them, and also from subjects with foul breath, a faint blue coloration is obtained in the starch iodide solution used for collecting the iodine liberated from the pentoxide when, and only when, more than 1 liter of expired air is passed through the analyzer train;[17] this amount is five to ten times that used in the analyses reported here.

The concentrations of alcohol in the bloods of the one hundred subjects used in determining the coefficient of distribution ranged between 0.22 and 2.88 mg. per cubic centimeter. The concentration of alcohol in the venous air was determined simultaneously with that in the blood. The values calculated for the coefficients of distribution of alcohol between air and blood are shown as dots in Fig. 7. The average for the series was 1:1,307, with extremes of 1:1,180 to 1:1,307, variations of −9.7 and +8.9 per cent.

Harger, Lamb, and Hulpieu[1] have reported a similar series of determinations in which the "drunkometer" was used to obtain the concentration of alcohol in the expired air from which that in the alveolar was calculated on the basis of the per cents of carbon dioxide in expired and alveolar air. The values they obtained are shown in Fig. 7 as circles; the average value is approximately 2,000, but the extremes are 1:1,220 to 1:4,200, variations of −39 and +110 per cent. Such variations would appear extreme even for medicolegal approximations.

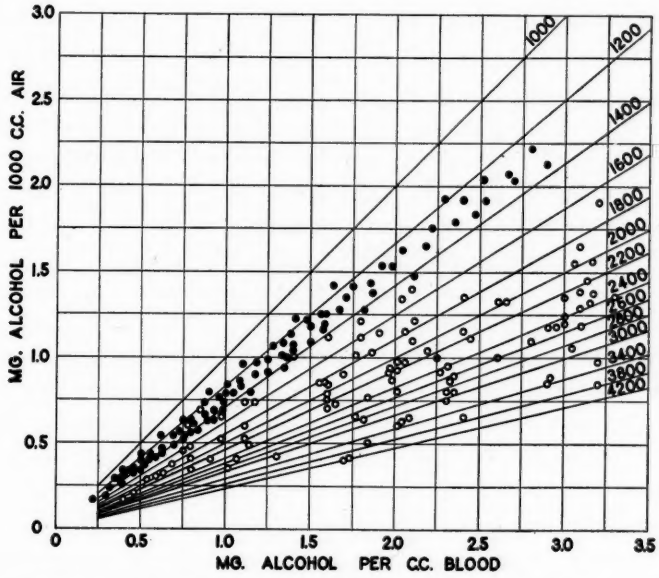

Fig. 7.—The coefficient of distribution of alcohol between blood and lung air. Dots, values as determined here; circles, values as reported by Harger, Lamb, and Hulpieu.

7. TEMPERATURE AT WHICH THE DISTRIBUTION OF ALCOHOL OCCURS IN THE LUNGS

The coefficient of distribution of 1:1,307, as given in the previous section, corresponds to a temperature for the distribution of 38° C. (see Fig. 2). This temperature is very nearly that of the blood flowing through the lungs.[18, 19] No measurements have ever been made of the temperature of the air in the lungs, and few reliable ones have been made of the temperature of the air leaving the lungs when not mixed with air in the respiratory dead space. The most recent and precise are those made by Seeley.[20] He finds that when the air breathed is at 20° C., the temperature of the exhaled air measured at the larynx is 35° C.; when the air breathed is 10° C., the temperature of the exhaled air falls to 34° C., and when breathed at 40° C. it rises to 36° C. It is certain that the temperature of the expired air does not precisely represent that in the lungs. If it did, the air in the alveolar spaces would be indicated as having a lower temperature than the blood passing through the lungs.

The air in the respiratory dead space, which is motionless between the times of inhalation and exhalation, comes into partial equilibrium, as we have shown, with the alcohol on the surface of the respiratory passages; this alcohol appears

in the air of the first part of expiration. It is improbable, for reasons which we have previously indicated, that during expiration the rapidly moving alveolar air either receives or gives up alcohol to surface tissues which may be at a lower temperature than the lungs. We have, however, investigated this point. On rebreathing from the tube of the apparatus shown in Fig. 4, to obtain venous air, the inhaled air becomes warmed to nearly 40° C. We have already shown (Fig. 5) that the concentration of alcohol in the alveolar air, after inhaling air at ordinary room temperature, is nearly identical with that of the venous air. This fact suggests that the temperature of the expired air has no influence upon the concentration of alcohol in the alveolar air. These observations were extended to include room temperatures of 10° and 40° C. Neither temperature affected the concentration of alcohol, as determined in the alveolar air.

When the alveolar air was exhaled into the tube (apparatus Fig. 4), heated to 40° C. and allowed to remain there, no fog and no condensation occurred; when the temperature of the tube was lowered to 35° C., there was a slight, but definite, fog; and when the experiment was repeated at a temperature of 30° C., there was a heavy fog, with condensation of a film of water upon the interior of the tube. This observation indicates that the alveolar air is saturated with water vapor at a temperature above 35° C. If, then, during expiration, the air from the depths of the lungs does not lose water in the respiratory passages, even at their lower temperatures, it is highly probable that it would not lose alcohol.

More precise measurement of the water vapor was obtained by direct determination from the alveolar and venous airs. A U-tube containing 70 Gm. of anhydrous magnesium perchlorate was attached to the tube from stopcock *E* of the apparatus shown in Fig. 4. The glass connections were warmed with a heating coil to prevent condensation. Two hundred cubic centimeters of alveolar or venous air were drawn through the U-tube, and the gain in weight was determined. The values obtained for five determinations on one subject for alveolar air with inspired air at 20° C. were: 43.0, 43.1, 44.0, 42.6, and 43.2 mg. per liter, the average of which corresponds to the amount of water in air fully saturated at a temperature of 37.2° C. The values obtained from the venous air with the rebreathed air at 40° C. were: 41.7, 44.2, 43.2, 44.0, and 43.4 mg. per liter of air.

CONCLUSIONS

1. The coefficient of distribution of alcohol between air and blood, now widely employed, is that assigned by Harger, Lamb, and Hulpieu, viz., 1:2,000. We find that the correct value, as determined both in vitro and in vivo, is 1:1,300.

2. The discrepancy is, in part, due to the loss of alcohol in condensed water vapor in containers used to collect expired air. This loss has been demonstrated.

3. The concentration of alcohol in lung air cannot be correctly calculated from the concentration in mixed expired air on the basis of carbon dioxide content of these airs. Alcohol diffuses more rapidly into the air of the respiratory dead space than does carbon dioxide. The diffusion does not affect the concentration in the alveolar or venous air.

4. The concentration of alcohol in the venous air corresponds closely to that in the alveolar air. A method is described for obtaining venous and alveolar air without loss of alcohol.

5. The temperature at which the distribution of alcohol between air and blood takes place in the body is that of the blood in the lungs.

6. The procedure now widely used in medicolegal investigation and statistical studies for determining the concentration of alcohol in the blood from that in the expired air is subject to serious errors. It is, however, possible to determine accurately the concentration in the blood from that in the lung air.

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A CLINICAL METHOD FOR THE DETERMINATION OF ASCORBIC ACID IN BLOOD PLASMA AND URINE*

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MINDLIN and Butler[1] have developed a method for the determination of plasma ascorbic acid, measuring photoelectrically the excess dye remaining after the reaction between ascorbic acid in a metaphosphoric acid filtrate and 2,6 dichlorophenolindophenol. In avoiding a titrimetric procedure in strong acid solution which allows considerable error due both to slow reduction by other materials and to decomposition of the oxidized dye, the method represents a real advance. The method of Bessey[2] extends the usefulness of this procedure by eliminating the necessity of absolutely clear and colorless filtrates. The preparation of such filtrates in the first method requires considerable care and time in filtering; in the second method two readings of the photoelectric device are necessary for each sample. In both procedures the photoelectric readings must be completed immediately after each addition of dye, with due care exercised in observing possible "drifts" of the reading due to the presence of slowly reducing materials, all of which is very time-consuming in serial analysis. The finding of Bukatsch[3] that oxidized 2,6 dichlorophenolindophenol can be quantitatively extracted from acid solution with xylene has led to an accurate, rapid clinical method. The method developed includes all the advantages of the procedures described, does not require special care in the preparation of the filtrate, and requires only a single photoelectric reading for each sample. The dye is allowed contact with the ascorbic acid for the short time necessary for reaction, then extracted into xylene, where it is no longer subjected to an acid medium or to slowly reducing substances, and is, therefore, completely stable for hours. The extraction involves a simple shaking with xylene and brief centrifugation. Because of the stability feature, as many samples as desired can be treated with the dye and extracted, and when all are completed, the color of the xylene layers measured as a unit in the photoelectric device. The separation of the chemical work from the photoelectric measurements saves time in its greater convenience. The stability of the dye in xylene permits the reliable use of the visual colorimeter in the absence of photoelectric devices.

The method to be described was developed with the view of increasing the ease and speed of plasma ascorbic acid determinations in serial analysis required in a recent research project.

DETERMINATION

Apparatus and Reagents.—Any photoelectric colorimeter, or, as employed in this work, a Coleman "DM" photoelectric spectrophotometer.

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Metaphosphoric acid. Six grams of clear sticks of glacial metaphosphoric acid, dissolved in 100 c.c. of water and filtered. This reagent is serviceable for eight to ten days if kept in the icebox.

Dye solution. Approximately 12 mg. of 2,6 dichlorophenolindophenol dissolved in 200 c.c. of warm distilled water, cooled and filtered. This reagent is satisfactory for eight to ten days if kept in the icebox.

Sodium hydroxide. An approximately 0.8 N solution made by dissolving 32 Gm. of sodium hydroxide in 1 liter of water.

Phosphate-citrate buffer. Approximately pH 4.0. Mix 30 c.c. of 0.2 M disodium phosphate and 50 c.c. of 0.1 M citric acid.

Bromcresol green 0.04 per cent. Add 14.3 c.c. of 0.01 N sodium hydroxide to 0.1 Gm. of bromcresol green and make up to 250 c.c. with water.

Xylene, C.P.

Method.—Blood for analysis should be collected (approximately 7 c.c.) with a dry syringe and kept in thoroughly clean pyrex tubes containing oxalate (about 20 mg.) as an anticoagulant. Excessive shaking and other causes of hemolysis are to be avoided. The tubes are chilled after collection and analysis is started within five hours. The blood is centrifuged and the plasma is withdrawn. Three cubic centimeters of plasma are measured into a test tube, followed by 3 c.c. of distilled water and 6 c.c. of 6 per cent metaphosphoric acid. After thorough mixing and allowing to stand for fifteen minutes, the tubes are centrifuged at 2,500 r.p.m. for fifteen minutes. The metaphosphoric acid filtrate may be kept for several hours in the cold without loss of ascorbic acid. A pipette is inserted into the supernatant fluid and exactly 8.0 c.c. of filtrate are removed to a 30 c.c. test tube. Two drops of bromcresol green indicator are added and 0.8 N sodium hydroxide is added dropwise with shaking until a green color is reached, then approximately 1 c.c. of the phosphate citrate buffer is added. Exactly 2.0 c.c. of the dye solution are added, the tube is revolved for mixing, the solution is layered with 12.0 c.c. of xylene, corked, and shaken for ten to fifteen seconds. Only fifteen to thirty seconds need elapse between addition of the dye and extraction in order to avoid slow, interfering reactions. The amount of dye prescribed in this test is sufficient to react with approximately 0.05 mg. of ascorbic acid or a plasma ascorbic acid of 2.5 mg. per cent. If more than this is encountered and the dye is completely reduced, accuracy is not sacrificed by adding a further 1.0 or 2.0 c.c. of dye at this stage. The tube is centrifuged briefly to separate the layers. Occasionally, and more often on too vigorous a shaking, the two layers do not separate well. In this case, after the first centrifugation, the xylene layer should be stirred briefly with a glass rod and again centrifuged. At the end of a series of analyses a "control" tube should be prepared, containing 8 c.c. of water, 1 c.c. of buffer, and exactly 2.0 c.c. of dye, followed by xylene and extraction.

The xylene layer may now be poured off easily and at any time within several hours into the special cells of the photoelectric device. In the case of the spectrophotometer, with xylene in the "solvent" tube, the transmission of the "unknown" and "control" tubes are determined at wave length $\lambda = 500\ \mu\mu$. (In the Evelyn or other photoelectric colorimeters the filter trans-

mitting maximally at 500 mμ should be used.) The difference in the logarithms of these two transmissions is directly proportional to the amount of ascorbic acid originally present, that is,

$$C = k(\log {}^{I_0}/I \text{ control} - \log {}^{I_0}/I \text{ unknown}) = k(\triangle \log).$$

Entirely similar relations are given in Bessey's paper for the case of the Evelyn colorimeter. The value of K must be determined only once for the prescribed conditions and for the instrument used, with a carefully weighed or iodine standardized ascorbic acid solution. Thus in this case a log = 0.064 was equivalent to 0.01 mg. of ascorbic acid, hence the milligrams of vitamin C involved in a given determination would be

$$C = \frac{\triangle \log}{0.064} \times 0.01.$$

Since the 8 c.c. of filtrate represents 2.0 c.c. of plasma, then in milligrams per cent of plasma ascorbic acid

$$C = \frac{\triangle \log}{0.064} \times 0.01 \times 50 = 7.81 \ (\triangle \log).$$

If the visual colorimeter is used, various dye controls must be prepared as above in order to permit accurate comparison with the widely different ascorbic acid values encountered in routine analysis. Also, each dye solution utilized must be standardized against a carefully prepared ascorbic acid solution. If reduction of the amount of dye prescribed is great, more favorable colorimetry is obtained by adding a greater excess of dye. Visual colorimetry cannot, however, provide the accuracy obtained by photoelectric measurement.

TABLE I

REPRODUCIBILITY OF METHOD AND COMPARISON WITH BESSEY'S[2] PROCEDURE OF PLASMA ASCORBIC ACID DETERMINATION

SAMPLE	PRESENT METHOD (MG. %)	BESSEY'S METHOD (MG. %)	AVERAGE % DIFFERENCE
Blood 1	0.74	0.77	4.6
	0.70	0.72	
	0.71	0.76	
Blood 2	1.08	1.16	4.3
	1.06	1.12	
	1.11	1.11	
Blood 3	0.38	0.41	5.0
	0.42	0.44	
	0.39	0.40	
Blood 4*	1.79	1.83	2.8
	1.82	1.88	
	1.82	1.87	

*Taken after administration of vitamin C.

The same method may be readily applied to urine. Equal volumes of freshly voided urine and 6 per cent metaphosphoric acid are mixed and filtered. An appropriate volume of this filtrate to be used may be judged by a rough titration into 2 c.c. of the dye.

The reliability of the indophenol dye-ascorbic acid reaction and recoveries obtained by its use have been amply shown.[1, 2] It was necessary only to check on the quantitative extraction of the oxidized dye from acid solution by xylene and the applicability of Beer's law to the xylene solution of dye. Both of these stipulations were satisfied in the concentration range of dye utilized in this test. Table I illustrates the agreement of the values found on several samples of blood by Bessey's method[2] and the present modification.

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A SIMPLE RAPID METHOD FOR DETERMINING RELATIVE BLOOD VOLUME CHANGES BY SPECIFIC GRAVITY STUDIES*

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RECENT advances in the study and treatment of shock have made necessary a simple practical method for determining changes in blood volume during the development and treatment of that condition. We present herein a method which is thought to represent such a procedure based entirely on specific gravity determinations.

Methods that are at present in use for estimating blood volume changes uniformly depend upon the determination of the concentration of various substances in the circulating blood, and from the known total amount of that particular substance in the blood, calculating the total volume of blood therefrom. With this general principle as a common feature there are two separate, practical approaches that may be utilized. The first of these is the dye method, first introduced in 1915 by Keith, Rowntree, and Geraghty.[1] These investigators determined the blood volume from the concentration of vital red injected into the circulating blood. The most significant modification of this method has been that of Gregersen, Gibson, and Stead,[2] who introduced Evan's blue as the dye and determined the dye concentration by photoelectric colorimetry. Although there are disadvantages and sources of error in these methods, their use has become well established in recent years. It is, however, with the second of these general methods for determining blood volume changes that the present consideration is particularly concerned. This method takes into account the changes in concentration of red blood cells, hemoglobin, or plasma proteins. Kottman[3] in 1906 was the first to utilize this principle. In more recent years a number of investigators have studied blood volume changes, particularly after injection of isotonic and hypertonic fluids, using hemoglobin changes as the basis of blood volume alternations. Most of these workers have used difficult gasometric methods for hemoglobin determination. Bogert, Underhill, and Mendel[4] showed that the variations in hemoglobin were an accurate indication of relative blood volume change by simultaneous determinations of total solids of the blood. Smith and Mendel,[5] Blalock, Beard, and Thuss,[6] Miller and Poindexter,[7] and Robertson[8] obtained apparently good results in estimating

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blood volume change by hemoglobin alterations after intravenous injections of fluids in animals. Gilligan, Altschule, and Volk[9] used changes in the hematocrit to calculate changes in blood volume in human beings following intravenous injections of various fluids. These investigators were able to compare the results of this method with the dye method of Gregersen, Gibson, and Stead[2] in a few cases. They found the results to be in good accord.

Investigators, particularly Lamson and Rosenthal[10] and Miller and Poindexter,[7] have seriously objected to the older dye methods on the basis of inaccuracy. However, these workers failed to take into account the residual dye remaining from previous injections in repeated blood volume determinations. This source of error has been clarified by Smith.[11] The method of Gregersen, Gibson, and Stead[2] at present must be considered the most accurate means of determining a given blood volume level, but is not itself free from disadvantages and sources of error. Particularly in cases of shock and burns under clinical and experimental conditions, the curve of disappearance of the blue dye will be variable because of variable degrees of capillary damage. Under these same circumstances there will be relatively less disappearance of red blood cells from the circulating blood. Furthermore, in the use of the dye method for repeated blood volume estimations, the necessity of determining the disappearance curve in a particular subject is an almost insurmountable disadvantage in clinical shock and burns; to a less extent is this true under experimental conditions.

In studying the alterations of the blood in experimental shock in dogs, attention was called to the great practical value of estimating changes in blood volume by changes in the hematocrit. It was also found that the red blood cell percentage (hematocrit) could be accurately determined from the specific gravity of whole blood and plasma. This report is, therefore, designed to illustrate the simple and practical manner of determining relative blood volume changes from specific gravity determinations. The method is adapted only to the determination of approximate plasma volume changes, and not to the accurate determination of the blood volume level. The procedure is based on calculating the hematocrit from the specific gravity of whole blood and plasma, and determining from changes in the hematocrit, alterations in the plasma volume.

METHODS

Dogs were used in all experimental work. Anesthesia was accomplished by intravenous injection of nembutal. Blood samples were taken at varying intervals, an average of four samples for each experiment. Blood was withdrawn from exposed jugular veins so that no compression of the vein was necessary. Shock was produced by trauma to extremities, trauma to intestine, intraperitoneal injection of 25 per cent sodium chloride, or application of tourniquets to both legs with subsequent release of the tourniquets. The average duration of the experiments was nine hours. Simultaneously blood pressure was determined by mercury manometer connected with the carotid artery. Heparin was used as the anticoagulant. On all samples of blood red cell counts, hematocrits, specific gravity of whole blood, and specific gravity of the plasma were determined. The hematocrit was determined according to the method of Win-

trobe,[12] centrifugation being carried out for forty-five minutes at 3,000 r.p.m. The specific gravity of whole blood and plasma were determined by the Falling Drop Method of Barbour and Hamilton.[13]

PROCEDURE

The hematocrit may be determined by specific gravity determinations of blood if it is recognized that the specific gravity of the whole blood is dependent upon the specific gravity of the red blood cells, the specific gravity of the plasma, and the proportions of the latter two components. This fact is apparent since the specific gravity of a mixture of two solutions is proportionately dependent upon the specific gravities of the two solutions. Assuming that solution A is composed of 4 c.c. of solution B and 2 c.c. of solution C, then

$$(1)\ \text{Sp. Gr. of A} = \frac{(\text{Sp. Gr. of B} \times 4) + (\text{Sp. Gr. of C} \times 2)}{6}$$

The same will be true of whole blood, as follows:

$$(2)\ \text{Sp. Gr. of Whole Blood} = \frac{(\text{Sp. Gr. of R.B.C.} \times \text{R.B.C. \%}) + (\text{Sp. Gr. of Plasma} \times 100 - \text{R.B.C. \%})}{100}$$

Solving for red blood cell percentage,

$$(3)\ \text{R.B.C. \% (Hematocrit)} = \frac{(\text{Sp. Gr. of Whole Blood} \times 100) - (\text{Sp. Gr. of Plasma} \times 100)}{\text{Sp. Gr. of R.B.C.} - \text{Sp. Gr. of Plasma}}$$

It is at once obvious that for the hematocrit to be determined from equation (3), the specific gravity of whole blood, of plasma, and of the red blood cells must be known. The latter factor should be a constant, and the former two factors should be readily determinable by the Falling Drop Method. It is an easy matter to ascertain the specific gravity of red blood cells from equation (2) if the specific gravity of whole blood, specific gravity of plasma, and hematocrit are known:

$$(4)\ \text{Sp. Gr. of R.B.C.} = \frac{(\text{Sp. Gr. of Whole Blood} \times 100) - (\text{Sp. Gr. of Plasma} \times 100 - \text{Hematocrit})}{\text{Hematocrit}}$$

From a series of 32 blood samples shown in Table I, taken from ten dogs selected at random from a larger series, it has been possible to determine the mean specific gravity of red blood cells to be 1.0953, with a standard deviation of 0.00205. Substituting then, 1.0953 in equation (3), it is possible to solve for the hematocrit if the specific gravity of whole blood and specific gravity of plasma are known. Table I shows that the red blood cell percentage calculated by this means differs only slightly from the actual hematocrit. To facilitate the determination of the hematocrit from the specific gravity of the whole blood and of the plasma, a nomogram, Fig. 1, has been constructed.

In using the hematocrit to estimate blood volume changes, it is necessary to determine an original blood volume by the dye method or to assume an original blood volume based on body weight. Blood volume in dogs has been estimated as 0.0915 of body weight.. The total volume of red blood cells can then be determined from the hematocrit and the original blood volume:

Total R.B.C. Volume = Blood Volume (0.0915 of Body Wt.) × Hematocrit

Assuming this total volume of red blood cells to remain constant, the resulting

blood volume can then be calculated from the subsequent hematocrits as follows:

$$\text{Blood Volume (2)} = \frac{\text{Red Blood Cell Volume} \times 100}{\text{Hematocrit (2)}}$$

In order to simplify this estimation Fig. 2 represents a nomogram which may be used to determine quickly the blood volume resulting after change in the hematocrit.

TABLE I

SPECIFIC GRAVITY OF RED BLOOD CELLS AND RED BLOOD CELL PERCENTAGE CALCULATED FROM SPECIFIC GRAVITY OF WHOLE BLOOD AND PLASMA

DOG NO.	BLOOD SP. GR.	PLASMA SP. GR.	HEMATOCRIT	HEMATOCRIT CALCULATED	ERROR	ERROR PER CENT	R.B.C.* SP. GR.	REMARKS
5	1.0511	1.0248	36	37	1.0	2.6	1.0978	Leg traumatized but dog did not go into shock
	1.0525	1.0248	37.5	39	1.5	4.0	1.0989	
	1.0547	1.0274	39.5	40	0.5	1.3	1.0965	
	1.0552	1.0257	41	42	1.0	2.4	1.0976	
	1.0582	1.0275	45	45.5	0.5	1.1	1.0964	
11	1.0542	1.0231	42.5	43	0.5	1.2	1.0963	Control dog
12	1.0511	1.0250	37	37	0	0	1.0955	Leg traumatized; dog died from shock
	1.0526	1.0261	39	38.3	0.7	1.8	1.0938	
	1.0596	1.0276	47	47	0	0	1.0956	
	1.0615	1.0281	49	49	0	0	1.0962	
	1.0661	1.0286	53.5	55.7	2.2	4.1	1.0986	
14a	1.0479	1.0234	34.5	33	1.5	4.3	1.0944	Shock by manipulation of intestines
	1.0604	1.0253	50	50	0	0	1.0955	
16	1.0497	1.0212	39.7	38	1.7	4.3	1.0930	Shock not produced by trauma to intestines
	1.0487	1.0222	37	36	1.0	2.5	1.0938	
	1.0501	1.0228	37.7	38	0.3	0.8	1.0949	
	1.0531	1.0235	39.7	41	1.3	3.5	1.0981	
	1.0537	1.0239	42	42	0	0	1.0948	
18	1.0488	1.0215	34.9	36.5	1.6	4.6	1.0940	Dog shocked by trauma to intestines
	1.0521	1.0221	40.2	41	0.8	2.0	1.0967	
	1.0512	1.0225	37.7	39	1.3	3.5	1.0986	
	1.0635	1.0252	53.5	54	0.5	0.9	1.0968	
17	1.0470	1.0181	37.8	37	0.8	2.1	1.0945	Dog died from nembutal
	1.0531	1.0200	43.5	44	0.5	1.1	1.0963	
20	1.0450	1.0211	33.5	32	1.5	4.5	1.0924	Shocked by sodium chloride intraperitoneally
	1.0514	1.0220	39	40	1.0	2.6	1.0974	
23	1.0418	1.0240	26.6	24	2.6	9.8	1.0910	Shock by tourniquet to legs
	1.0535	1.0278	40	38	2.0	5.0	1.0920	
	1.0583	1.0281	45.8	45	0.8	1.7	1.0946	
26	1.0519	1.0233	42	39.7	2.3	5.5	1.0914	Shock by tourniquet to legs
	1.0567	1.0265	46	44	2.0	4.3	1.0920	
	1.0729	1.0311	67	64	3.0	4.5	1.0935	

*Mean specific gravity of red blood cells—1.0953.
Standard deviation—0.0020 or 0.2053 per cent.

RESULTS

Using the principles indicated above, it is possible to determine relative blood volume changes in a time as short as ten minutes, most of which time is required for a short centrifugation of the blood sample in order that a small amount of plasma may be obtained for specific gravity determination. In order to illustrate the manner of application and the relative accuracy of the present method, data on six dogs selected from a larger series are presented in Table II. It will be seen from the table that there is a close correlation between

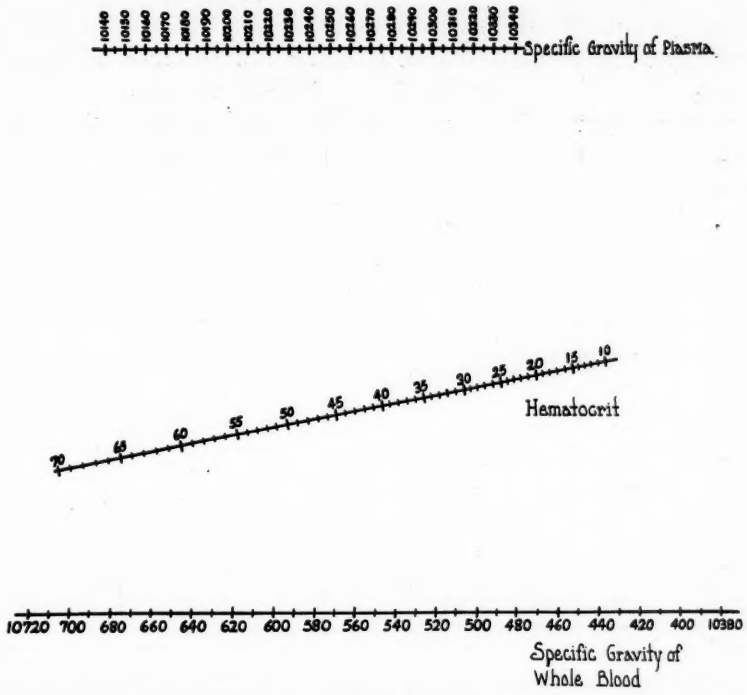

Fig. 1.—Nomogram for determining hematocrit from specific gravity of whole blood and plasma. A straight line from the specific gravity of whole blood and plasma crosses the middle line at the hematocrit level.

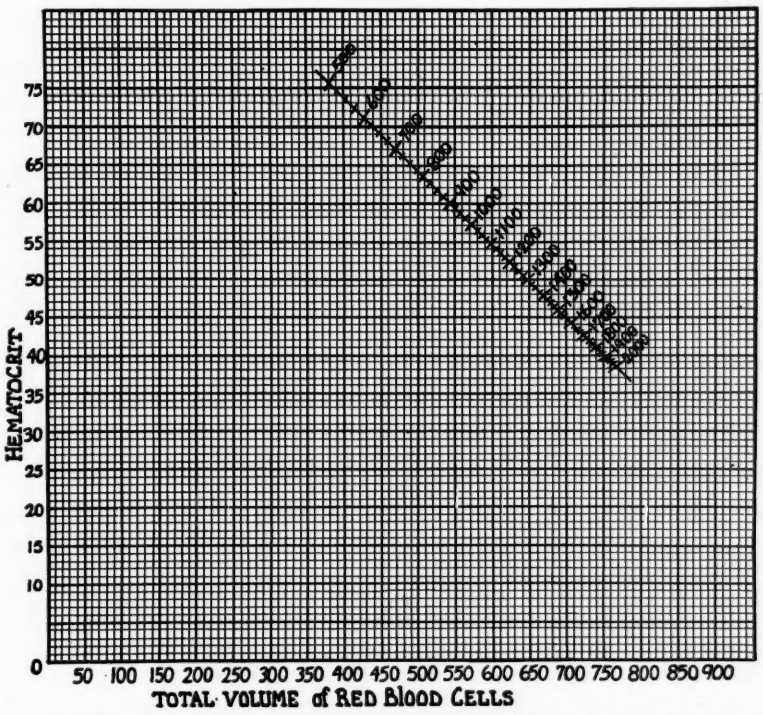

Fig. 2.—Nomogram for determining the blood volume changes from the hematocrit. A straight line from point 0 to the blood volume crosses the horizontal line of the original hematocrit at a point where the latter is intersected by the vertical line of the red blood cell volume. Then a line drawn from 0 through the point of intersection of red blood cell volume and subsequent hematocrits, crosses the blood volume indicator at the point of resulting blood volume.

TABLE II

BLOOD VOLUME CHANGES IN EXPERIMENTAL SHOCK AS CALCULATED FROM SPECIFIC GRAVITY OF WHOLE BLOOD AND PLASMA

DOG NO.	BLOOD SP. GR.	PLASMA SP. GR.	HEMATOCRIT	R.B.C. % CALCULATED	BLOOD VOLUME	MAXIMUM DECREASE OF BLOOD VOLUME	EXPECTED DECREASE (3% BODY WEIGHT)	REMARKS
12	1.0511	1.0250	37	37	830*	270	273	Shock by trauma to legs
Wt.	1.0526	1.0261	39	38.3	800			
9.1	1.0596	1.0276	47	47	660			
kg.	1.0615	1.0281	49	49	635			
	1.0661	1.0286	53.5	55.7	560			
15	1.0517	1.0231	38.7	39	1140*	405	375	Shock by trauma to intestines
Wt.	1.0553	1.0244	43	43.5	1025			
12½	1.0634	1.0271	52	52.5	840			
kg.	1.0688	1.0276	59	60.5	735			
19	1.0538	1.0241	41	41.5	915*	435	(720-250) 470	250 c.c. of 25% sodium chloride intraperitoneally. 720 c.c. fluid in peritoneum at autopsy
Wt.	1.0619	1.0240	49	52.5	720			
10	1.0741	1.0258	65	68	480			
kg.								
20	1.0451	1.0211	33.5	32	830*	355	(600-225) 375	225 c.c. of 25% sodium chloride intraperitoneally. 600 c.c. of fluid in peritoneum at autopsy
Wt.	1.0514	1.0220	39	40	660			
9.1	1.0596	1.0226	48.6	50	520			
kg.	1.0654	1.0265	53.9	55.5	475			
21	1.0517	1.0215	39	40	1080*	410	(700-250) 450	250 c.c. of 25% sodium chloride intraperitoneally. 700 c.c. in peritoneum at autopsy
Wt.	1.0655	1.0220	56	57.5	755			
12	1.0714	1.0248	68	65	670			
kg.								
25	1.0547	1.0266	41.2	41	710*	220	230	Shock by tourniquets to legs for 4½ hours
Wt.	1.0694	1.0298	61	60	490			
7.7	1.0693	1.0294	60.5	60	490			
kg.								

*Blood volume calculated as 0.0915 of body weight.

the actual hematocrit and the calculated hematocrit. In almost all instances the difference is not greater than the error to be expected in hematocrit determinations. Since in shock the degree of capillary damage is such that colloidal dyes escape irregularly from the circulating blood, it is not possible to check the results of this method by the usual dye methods of blood volume determination. Magladery, Solandt, and Best[14] have recently pointed out the inadequacy of dye methods for blood volume determination in shock. It will, however, be noted that there is close agreement between the blood volume decrease based on these hematocrit values and the blood volume decrease expected to occur in the development of shock. This expected decrease is based on the body weight of the animals, 3 per cent of body weight or one-third of blood volume being expected to be lost when the duration of the experiment is as long as eight hours.[15] The closest possible check on the method is afforded by the intraperitoneal injection of 25 per cent sodium chloride solution. Presumably all the lost plasma will be in the peritoneal cavity where it may be measured. In three dogs shown, Nos. 19, 20, and 21, it will be seen that there is accurate correlation in this regard.

DISCUSSION

In all circumstances where blood volume alteration due to increase or decrease of plasma volume occurs, these changes may be simply and accurately determined from the specific gravity of whole blood and plasma. Intermediately the hematocrit is calculated. Naturally, in those conditions where blood volume decrease is attended by an appreciable loss of red blood cells, the method will be of considerably less value. It should be remembered, however, that in those conditions where there is loss of red blood cells, the dye method will also be in error because of dye being lost from the blood stream. It would seem that wherever repeated estimations of blood volume may be accomplished by the indirect dye method, the relative blood volume changes may be computed from the specific gravity.

In experimental shock due to mild trauma to extremities, application of tourniquets, trauma to intestine, or intraperitoneal injection of hypertonic sodium chloride solutions, the method is particularly applicable. This is true because the loss of red blood cells is negligible under these circumstances.

In human beings the same methods should be applicable for determining relative blood volume changes. In a series of determinations of specific gravities of human red blood cells somewhat lower values than those for dogs have been obtained. The mean specific gravity of human red blood cells was found to be 1.0913, with a standard deviation of 0.0016. The formula for determining the red blood cell percentage (hematocrit) in man would then be:

$$\text{Hematocrit} = \frac{(\text{Sp. Gr. of Whole Blood} \times 100) - (\text{Sp. Gr. of Plasma} \times 100)}{1.0913 - (\text{Sp. Gr. of Plasma})}$$

Clinically, relative blood volume changes calculated from specific gravity of whole blood and plasma might be expected to be accurate in a great many circumstances. In cases of burn, where the decreased blood volume is due entirely to escape of plasma, the method should be particularly useful. This is especially true since most all cases of burn are seen quickly enough in hospitals that a sample of blood drawn on admission will reflect well the total red blood cell volume as calculated from the total blood volume determined from weight, and the red blood cell percentage determined from specific gravity of whole blood and of plasma. In the usual case of shock from trauma, operation, and anesthesia, intestinal obstruction, marked diarrhea, protracted vomiting, etc., as long as there is no excessive loss of blood, the method should be suitable. Where blood volume changes temporarily occur from injection of various isotonic and hypertonic solutions as well as whole or concentrated plasma, it will be very simple to determine these changes by changes in the specific gravity.

It should be pointed out that the specific gravity of the red blood cells of dogs and of human beings varies not only in different individuals but also in the same individual at different times. The degree of variation, however, which has so far been encountered, has not been sufficient to cause an appreciable error in the calculations. A subsequent report shall consider in greater detail the specific gravity of human red blood cells and the variation of this property under different circumstances.

SUMMARY

1. The hematocrit, or red blood cell percentage changes reflect accurately the plasma volume changes under circumstances where there is no appreciable loss of red blood cells.

2. A new, quick method for determining red blood cell percentage by specific gravity studies of whole blood and plasma is presented.

3. Blood volume changes in experimental shock due to mild trauma, intraperitoneal injections of hypertonic solutions of sodium chloride, or application and subsequent release of tourniquets may be accurately followed by this method.

4. Clinically the method should prove of value in studying the relative volume changes in shock due to trauma, operations and anesthesia, intestinal obstruction, severe diarrhea, and protracted vomiting. The same procedure should be an accurate guide to fluid therapy in shock and other conditions.

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DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEVULOSURIA, Study of Two Cases in Brothers, Jacobsen, V. C. Am. J. M. Sc. 200: 304, 1940.

Two cases are reported of so-called essential levulosuria in young Jewish brothers. They bring the total on record to 33. The condition appears to be a metabolic anomaly in which the organism exhibits a partial or complete inability to convert levulose into glycogen. This seems to be due not to a hepatic deficiency, but to increased permeability of the kidneys by the levulose molecule. This renal hypothesis does not postulate any inherent kidney disease, although in one case with paroxysmal hypertension there were signs of renal irritation. In both cases an approximately equal amount of glucose was excreted, but only at the same time that levulose was passing through the kidneys. Normal dextrose and levulose tolerance tests were obtained.

The clinical importance of the condition lies in its early recognition, before the patient is subjected to the rigors of a diabetic regimen which may severely handicap the growing child. The treatment is reassurance that he does not have diabetes mellitus, and a diet without special restrictions. So far as is known, the passage of levulose through the kidneys causes no harm, although continual observation of these interesting people throughout their lives may later alter this dictum in some degree.

SERUM, Significance of Albumin-Globulin Ratio of, Melnick, D., Field, H., Jr., and Parnall, C. G., Jr. Arch. Int. Med. 66: 295, 1940.

The albumin-globulin ratios characteristic of normal and hypoproteinemic sera represent the relative amounts of two independent protein systems, which may be separated by salting-out procedures. Dissociation and association phenomena attributed to the serum protein molecules must occur within these independent systems. Exposing normal and hypoproteinemic sera to the same environmental factors by dialysis of one against the other does not alter the ratio of one protein fraction to another. The influence of incipient denaturation on the salting-out behavior of the serum protein complex has been studied and serves to explain the alleged conversion of albumin to globulin reported by others. Urinary protein does not represent total serum but is derived principally from the albumin fraction.

The inadequacy of colloid osmotic pressure measurements in vitro and of the determinations of albumin and globulin for predicting the true oncotic pressure in vivo are discussed in the light of recent physicochemical studies of the serum protein complex.

PNEUMOCOCCI, Immune Reactions of Carriers and Non-Carriers of Type-Specific, Finland, M., Brown, J. W., and Barnes, M. W. Am. J. Hyg. 32: 24, 1940.

Cultural and serologic studies were carried out in a selected group of the personnel of the medical wards of a general hospital in which large numbers of patients with pneumonia are admitted for treatment. The studies were directed primarily to the isolation and identification of pneumococci and their types and the determination of type-specific pneumococcus antibodies.

The most efficient method found for detecting pneumococcus carriers consists of inoculating a swab of the pharynx into suitable culture media, injecting some of the growth resulting after four to six hours' incubation into a mouse and then examining the peritoneal washings after six to twenty-four hours.

Fifty-six per cent of the persons studied were found to harbor pneumococci in their pharynx on one or more occasions. The percentage of carriers detected was related to the

number of times the cultures were made. However, those in whom pneumococci were not found in the first culture were less likely to have them in subsequent cultures and, if pneumococci were acquired, they were more readily lost. The duration of the carrier state varied considerably.

The finding of pneumococci during this study was apparently unaffected by the recent presence of acute respiratory infections.

The distribution of pneumococcus types encountered showed a predominance of some types, such as III, VI, and XIII, which are usually frequent among normal healthy carriers. Twenty-six different specific types were encountered, and only 1 per cent of the strains of pneumococci failed to react with serums for types I to XXXII. In some instances mouse passage was necessary before the type could be determined.

Carriers of pneumococci frequently had, or acquired, homologous type-specific antibodies. The time of appearance of such antibodies in relation to the finding of the pneumococci, and the length of time over which the antibodies could be detected varied widely and was not always related to the duration of the carrier state.

Some indirect evidence was adduced which indicated that certain of the personnel acquired some of their pneumococci from patients under their care. In many instances, however, the findings suggested that the carriers acquired the various pneumococci from one another.

Four of the patients developed atypical pneumonia in the course of this study. Two of these were known to be chronic carriers, and the same types were found during their illness; one had a few pneumococci in his sputum during his illness but none before or later; and the fourth was a transient carrier before the onset of his pneumonia, but no pneumococci could be found during his illness. None of these four patients developed significant antibodies either to the pneumococcus which was found or to most of the common types associated with pneumonia.

These findings lend further support to the antigenicity of type-specific pneumococci in apparently healthy carriers.

The findings in the patients who developed pneumonia indicate that pneumococci found in patients with atypical pneumonias may not be etiologically related to the infection.

TISSUE: Improved Method of Staining Within Tissues, Leptotriches of Parinaud's Conjunctivitis and Gram-Positive Micro-Organisms, Verhoeff, F. H. J. A. M. A. 115: 1546, 1940.

Fix fresh tissue in Zenker's solution or solution of formaldehyde.

Embed in celloidin or paraffin. Celloidin sections are the more readily manipulated.

Cut sections as thin as possible, not more than 10 microns in thickness.

Stain sections in hematoxylin and eosin.

Mount, examine, and select sections that contain the largest foci of macrophages.

By the aid of xylene remove the selected sections from the slide and wash in oil of thyme, then in 95 per cent alcohol, and then in water. (Hematoxylin may be removed by acid alcohol, but this is not necessary.) If large foci of macrophages have been found, adjacent unstained sections will undoubtedly also contain them and may, therefore, be used instead.

The solvents now to be used are placed in small receptacles, such as ordinary salt cellars. Celloidin sections are to be immersed in these. In the case of paraffin sections the solutions are to be dropped on the slides by means of eye droppers. Water is placed in large finger bowls.

Place the celloidin section from the water on a slide. Wipe away any excess of water and drop on the section Stirling's crystal violet (methyl rosaniline 5 Gm., 95 per cent ethyl alcohol 10 c.c., aniline 2 c.c., water 88 c.c.) for two minutes.

Float the section from the slide into a bowl of water. Wash in several changes of water; this requires about two minutes.

Immerse in compound solution of iodine, from twenty to sixty seconds (iodine 1 Gm., potassium iodide 2 Gm., water 100 c.c.).

Wash in water and transfer to 95 per cent alcohol. Stir the section around until the stain begins to come out freely, about five seconds.

Transfer quickly to trichlorethylene. Stir the section around until the color ceases to come out freely, about ten seconds.

Transfer to oil of thyme one minute, then to 95 per cent alcohol for five seconds, and again to oil of thyme. Examine on the slide under the low power of the microscope. If precipitates are present, or differentiation is insufficient, differentiate further by alternate immersions in 95 per cent alcohol and oil of thyme, five seconds each.

Wash in xylene, two changes, and mount in cedar oil on a slide.

By this method the leptotriches should be deeply stained and easily recognizable when occurring, as is usually the case, in masses. Isolated leptotriches will probably not be recognized by inexperienced observers. If no leptotriches are visible, other structures, such as fibrin, granules of mast cells, and chromatin, may possibly be mistaken for them by those who have never seen these microorganisms. When leptotriches are present in masses, however, their appearance is so characteristic and so different from any other structures in the tissues that they are easily identified.

PNEUMONIA, The Sabin Agglutination Test and the Polysaccharide Skin Test (Francis) as Indices of Recovery in, Fox, W. W., Rosi, R., and Winters, W. L. Am J. M. Sc. **200:** 649, 1940.

The authors conclude from their observations that the agglutinin test is a reliable index to the development of increased resistance to the pneumococcus in the course of pneumonia. It is such a simple test that it may be readily used as a control for sulfapyridine treatment. It rarely becomes positive before the eighth day of the disease; and since in complicated cases it is delayed until the twelfth to thirteenth day, it is particularly important that sulfapyridine be continued longer in such cases. Relapse and late septic complications may be prevented if this is done.

The Francis test is reliable in only 50 per cent of the cases (with the polysaccharide solutions used). The solutions of polysaccharide are available only for the more common types.

A change from negative to positive Francis test occurs on an average of two days before the agglutinin test becomes strongly positive. When this change is noted, it is probably safe to decrease the dosage of sulfapyridine, and to discontinue it two days later.

As concluded in a previous paper, recovery in pneumonia treated with sulfapyridine depends on three factors: an adequate early leucocytosis, an adequately maintained sulfapyridine blood level, and development of active immunity by the patient for the type of pneumococcus causing his pneumonia. The agglutinin test is a simple, inexpensive, rapid method for determining the development of active immunity.

INTOXICATION, Use of the Urine in the Chemical Test for, Haggard, H. W., Greenberg, L. A., and Miller, R. P. J. A. M. A. **115:** 1680, 1940.

In the medicolegal diagnosis of intoxication the concentration of alcohol in the blood is frequently estimated indirectly from that found in the urine.

Unless special precautions are taken, serious errors may occasionally occur.

The ratio of the concentration of alcohol in blood to that of urine varies only slightly with the specific gravity of the urine; the ratio as determined is 1:1.3.

Absorption of alcohol from the bladder into the blood does not occur to a significant extent of the concentration found in the urine after drinking alcohol.

PREGNANDIOL EXCRETION, Diagnostic Value of, in Pregnancy Disorders, Cope, C. L. Brit. M. J. **2:** 545, 1940.

Pregnanediol is a product of corpus luteum and placental metabolism. Its excretion in the urine is relatively easily measured in any biochemical laboratory. An attempt has been

made to assess the diagnostic value of pregnanediol excretion in abnormal pregnancy. Its complete absence is nearly always evidence of serious abnormality, suggesting in early pregnancy the death of the fetus. The significance of a low pregnanediol excretion still remains uncertain. A normal pregnanediol excretion is not evidence that abortion will not occur or that the fetus is still alive. Both chronic nephritis and toxemia of pregnancy may interfere with pregnanediol excretion. Deviations from the normal excretion in these conditions do not necessarily mean that the gestation is abnormal. Pregnanediol excretion seems assured of a place as a diagnostic aid in obstetric disorders.

BLOOD COAGULATION, Effect of Nicotinic Acid on, Calder, R. M., and Kerby, G. P. Am. J. M. Sc. **200**: 590, 1940.

Clinical experience in a significantly large group of patients with chronic brucellosis revealed that nicotinic acid possesses the property of promoting coagulation of the blood. These patients often develop subcutaneous hemorrhages after slight trauma, and their blood clots slowly, retraction is imperfect, and the yield of serum is low. All these defects were completely remedied by the administration of nicotinic acid.

These observations were extended to a small group of patients with other clinical states associated with bleeding, and the results were uniformly encouraging. Presumably, the hemorrhagic tendencies in all these patients rested on a toxic, infectious basis.

Experimental studies reported show that nicotinic acid does not duplicate the action of, nor can it be substituted for, any of the known factors involved in blood clotting (calcium, platelets, thromboplastic principle, prothrombin, or thrombin). However, when it is added in vitro to blood containing excessive amounts of antithrombin (heparinized blood or samples from dogs in peptone or anaphylactic shock), it does induce coagulation. That this effect is not due to nonspecific alteration of hydrogen-ion concentration was indicated by contrast of nicotinic acid with seven other acids, none of which was as active in this respect as was nicotinic acid.

The quantities of nicotinic acid employed in in vitro experiments were so enormous when compared with the doses used clinically, that it is not justifiable to assume that the mechanism of its effect in patients is necessarily a chemical neutralization of antithrombin, though such an explanation is possible. Certainly, in view of the small doses effective in patients, the results cannot be attributed to a nonspecific acid in effect. It would seem logical to conclude that nicotinic acid corrects a fundamental deficiency in the organism; or, by some pharmacodynamic action (e.g., stimulation of the liver), induces the formation either of more prothrombin or of less antithrombin.

Whatever the mechanism of its action may be, experience indicates that further trial of nicotinic acid in various hemorrhagic states is warranted.

PNEUMONIA, PNEUMOCOCCIC, Should Serum Be Used in Addition to Sulfapyridine? Dowling, H. F., Abernethy, T. J., and Hartman, C. R. J. A. M. A. **115**: 2125, 1940.

A series of cases of pneumonia caused by pneumococcus type I through type VIII have been alternated for treatment with sulfapyridine alone and with sulfapyridine plus specific antipneumococcus serum. The mortality rate was 12.5 per cent in the group receiving sulfapyridine alone, and 9.8 per cent in the group receiving serum plus sulfapyridine. If the type III cases are omitted, the corresponding figures are 9.2 and 4.5 per cent, respectively.

Serum seemed to be particularly valuable as an adjunct to sulfapyridine in patients over 40 years of age.

Crisis occurred more frequently and was more prompt in the patients receiving serum in addition to sulfapyridine.

It is suggested that serum and sulfapyridine both be given to patients over 40 years of age with pneumonia and to those who are in need of a prompt defervescence.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, 201 West Franklin Street, Richmond, Va.

The Methods of Ferment Investigation* (Section 1)

THE Methods of Ferment Investigation is intended to review our present knowledge of all ferment methods used in industry, biology, and medicine. The complete work will have about 3,500 pages and will contain 214 different articles written by 131 collaborators from various countries throughout the world. The book will be written entirely in German. Up to now only the first section of the book has appeared. A short introduction given by E. Bamann (Tübingen) and K. Myrbäck (Stockholm). The first subdivision deals with the substrates of ester-splitting ferments: subtrates of the lipases by E. Bamann, natural glycerides by K. H. Bauer (Leipzig), acetylcholine by R. Ammon (Königsberg), substrates of the tannases by O. T. Schmidt (Heidelberg), chlorophyll by H. Fischer (München), phosphoric acid esters by E. Hackenthal and M. Kobel (Berlin), phosphatides by S. Belfanti, A. Ercoli, and M. Francioli (Milan), phosphoric acid by T. Posternak (Genf), and sulfuric acid esters by E. Hackenthal and M. Kobel. The second subdivision discusses the carbohydrates and glycosides: sugar by E. L. Hirst (Bristol) and S. Peat (Birmingham), isolation of natural heterosides by J. Rabate (Paris), synthetic glycosides by H. Elsner (Berlin), and thioglycosides by F. Wrede (Berlin).

Despite the obvious fact that most of the authors tried to condense their material as far as possible, nearly all articles are written rather clearly and contain an enormous amount of useful literature references. For the moment, it is a disadvantage that all these references, which are cited only by name and number in the individual articles, are combined into one bibliography which will be published as the last section of the whole work.

—*Ernst Fischer.*

The Methods of Ferment Investigation† (Sections 2, 3, 4)

THE three recently published sections of the "Methods of Enzyme Investigations" contain more than 60 different articles by a large number of collaborators from various countries. The subdivision about carbohydrates and glycosides, started in the first section, is continued now by articles by A. Purr (Prag) about glycolysis and starch, by H. Elsner (Berlin) and co-workers about galactogen, fructosans, and chitin. Glucosans and zytans, ascorbic acid, and related substances are discussed by E. L. Hirt (Bristol) and S. Peat (Birmingham), polyuronides by H. Bock (Karlsruhe), chondroitin sulfuric acid by T. Soda (Tokyo), the phosphorus-free derivatives of the carbohydrates by H. K. Barrenscheen and J. Pany (Wien), and the phosphorus-containing derivatives by R. Robinson and M. G. Macfarlane (London). The next subdivision is formed by a lengthy contribution of W. Klein (Freiburg) about the nucleic acids and their derivatives and by some shorter chapters about the proteins and their derivatives by A. Schäffner (Prag), E. Abderhalden (Halle), and F. Leuthard (Zürich). The amides are discussed in an especially interesting article by C. G. Holmberg (Lund), the thiols and disulfides by T. Bersin (Marburg), and finally the nucleoproteins, lipoproteins, and glycoproteins by St. J. V. Przylecki (Warsaw).

The third section is mainly devoted to the description of the modern methods in enzyme investigations. K. Felix (Frankfurt) describes the use of enzymatic investigation for the determination of the chemical structure of substances of high molecular weight, while T. W.

*Die Methoden der Fermentforschung. Edited by Eugen Bamann, M.D., and Karl Myrbäck, M.D. Section 1 with 172 pages and 2 figures. Georg Thieme, Leipzig, 1940.

†Die Methoden der Fermentforschung. Edited by Eugen Bamann, M.D., and Karl Myrbäck, M.D. Section 2 with 303 pages and 6 figures. Section 3 with 392 pages and 249 figures. Section 4 with 407 pages and 177 figures. Georg Thieme, Leipzig, 1940.

Astbury (Leeds) and J. C. Derksen (Eindhoven) contributes three excellent chapters about x-ray spectrographic methods and their results. G. Kortüm and M. Kortüm-Seiler (Tübingen) discuss absorption spectrum, Raman spectrum, and fluorescence. Heyrovsky's polarographic methods for electrical potentials are described by R. Brdička (Prag), the magnetic methods by E. Müller (Jena), determination of viscosity by R. Mohr (Freiburg), and the ultracentrifuge and diffusion methods by O. Lamm (Uppsala). Short chapters about dielectric, kryoscopic, and osmotic methods for molecular weight determination are contributed by S. Arrhenius (Uppsala), D. Krüger (Berlin), and G. V. Schulz (Freiburg). L. Kofler (Innsbruck) describes microdetermination of the smelting point and sublimation methods.

The rest of the third section and the whole fourth section deals with the enzymatic reactions themselves. E. Brunius (Stockholm) describes the various nomographic methods, M. Steiner (Göttingen) the maintenance and testing of suitable conditions for enzymatic reactions, and J. Lehman (Goteburg) the redox potential. "The calculation of the free energy of biochemical reaction" by W. Franke (München) is well written and instructive. The various physical and physico-chemical methods are summarized in several short chapters by L. Heilmeyer (Jena), B. J. Krijgsman (Utrecht), P. Wulff (Frankfurt), D. Krüger (Berlin), and by K. Linderstrøm-Lang and H. Holter (Køpenhavn), while the manometric methods are treated in a longer chapter by F. Dickens (Newcastle). The next subdivision is devoted strictly to chemical methods, especially micro-analytic methods: alcohols, aldehydes, and acids by M. Steiner and O. Glemser (Göttingen); carbohydrates by H. K. Barrenscheen and J. Pany (Wien); colorimetric determination of carbohydrates by G. Haugard (Køpenhavn); purine bases by J. E. Jorpes (Stockholm); titration of the carboxyl and of the amino groups by W. Grassmann and P. Stadler (Dresden), and the volumetric and manometric determination of amino acids by the same authors. A short résumé of the more biological methods is given by E. Werle (Düsseldorf), while K. Linderstrøm-Lang and H. Holter (Køpenhavn) discuss very carefully the new histochemical methods. The last part of the fourth section contains the first chapters of the subdivision dealing with the isolation and testing of various emzymes. The general methods for preparation of enzyme-containing solutions are written up by H. Kraut and A. Weischer (Dortmund), the special methods for enzymes of invertebrates by B. J. Ktijgsman (Utrecht), and those of bacteria by H. C. Werkman and H. G. Wood (Ames, Iowa), up to now the only American co-workers. Methods for the concentration of enzyme preparations by evaporation are summarized by L. Vogel and P. Laverenz (München), and separation methods by means of freezing are described by J. Hartmann (München). The two final chapters are very interesting reports by H. M. Karström (Helsinki) about bacteria as material for enzymatic investigations.

Without doubt, these three sections of the "methods of enzyme investigations" contain an enormous amount of useful material for investigation in this and related fields. It is regrettable that due to the war conditions, some of the non-German authors were unable to read the proofs, since otherwise some apparent errors due to the translation of their original articles would have been eliminated.

—*Ernst Fischer.*

Bacteriology of Public Health*

DIFFERING from other bacteriology books in that it does not incorporate laboratory methods, this text presents facts of pathologic bacteriology from a public health viewpoint. This has the effect of permitting the student to become oriented without being confused by too much unnecessary information; it does not, however, mean that the matter is dealt with in a superficial manner. Indeed, the author is to be commended on carefully weeding out that which is extraneous. The emphasis throughout is on the bacteriology of disease rather than on bacteria which incidentally cause disease. Following a historical review, one finds chapters on drinking water and disease, on food idiosyncrasies, food poisoning, and food infections, and then a chapter devoted to each of the bacterial, virus, and protozoal diseases. The book, which is well written, includes a good bibliography and index.

*The Bacteriology of Public Health. By George M. Cameron, Ph.D., Associate Professor of Bacteriology, University of Tennessee. Illustrated. 451 pages. The C. V. Mosby Company, St. Louis, 1940.